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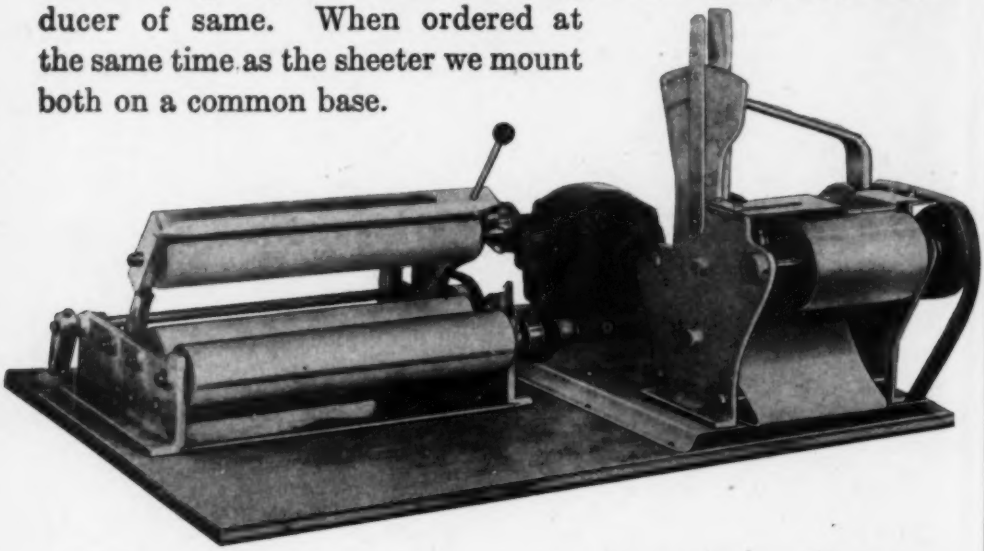

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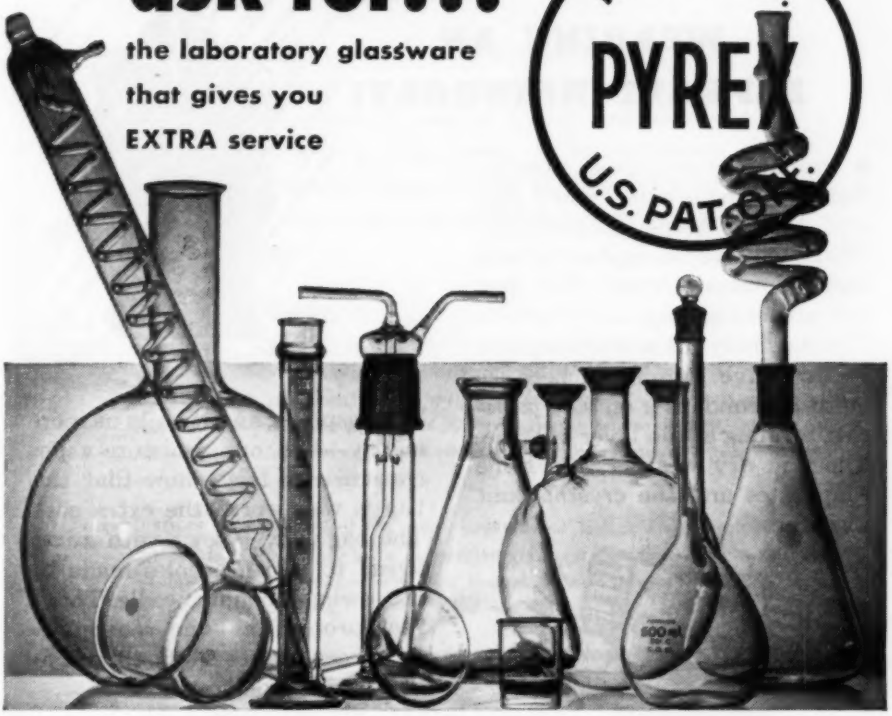

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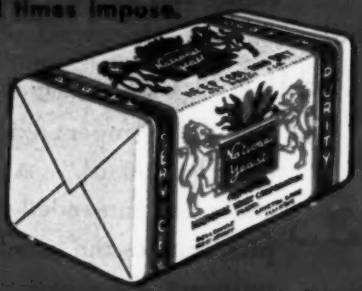

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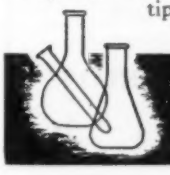

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CEREAL CHEMISTRY

VOL. XXV NOVEMBER, 1948 No. 6

EFFECT OF FORTIFICATION OF CANNED BREAD ON STABILITY [1]

SADIE BRENNER,[2] STUART G. DUNLOP,[3] and VIRGIL O. WODICKA[4]

ABSTRACT

The stability of thiamine, riboflavin, and niacin was investigated in canned bread during fermentation, baking, and storage. Synthetic and dried yeast sources of enrichment were used and the effect of each on baking quality of the flour and palatability of the finished bread measured. Riboflavin and niacin were retained almost completely during fermentation, baking, and storage. Thiamine, however, regardless of source, was decreased by approximately 15% during baking and 20 and 50% during 6 months' storage at 72° and 100°F. respectively. A 1% level of yeast (flour basis) was preferable to a 3% level insofar as acceptability and baking quality were concerned. The protein quality of the whole wheat and yeast bread was significantly superior to that of the white bread fortified with synthetic vitamins.

During World War II adequate amounts of thiamine, riboflavin, and niacin in rations were provided chiefly through the fortification of the biscuit and cracker components with dried yeast.[5] The low moisture content of the biscuit component insured against losses of labile thiamine during long periods of storage at elevated temperatures. However, due to the general unpopularity of the biscuits and crackers, endeavors were made to find other stable carriers for these vitamins. Since canned bread was being developed with the intention of partially or completely replacing the biscuit and cracker components, intensive studies were undertaken to investigate the possibilities of this universally well-liked item as a carrier of the B complex vitamins.

Materials and Methods

Fortification of canned bread with synthetic vitamins and natural sources were both investigated. The latter type of enrichment was

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[3] University of Colorado, School of Medicine, Denver, Colorado.
[4] Libby, McNeill and Libby, Blue Island, Illinois.
[5] The dried yeast used in biscuit fortification was specified to contain not less than 600, 600, and 1,000 μg. of thiamine, riboflavin, and niacin per gram of dried yeast respectively. Such concentrations were obtained by adding the synthetic nutrient to the live yeast cells prior to drying.

preferred as it supplied, in addition to thiamine, riboflavin, and niacin, the lesser known factors of the vitamin.B complex, thus producing a more complete and natural food. The natural sources used to provide the B complex vitamins were dried yeast (primary grown or debittered brewers') and whole wheat. Combinations of synthetic and natural sources were also used to determine if the natural enrichment would stabilize the synthetic enrichment.

Study 1. This study was undertaken to survey the potentialities of canned bread as a carrier for the vitamins of the B complex. It was conducted on the first test production run of the three contemplated types to be used in rations, namely, white, white with raisins, and whole wheat bread. Both white types of bread were fortified with synthetic vitamins.[6] Five cans of each type of bread were assayed initially and at each storage time interval (bimonthly) for thiamine, by the thiochrome procedure (8), and for riboflavin and niacin by the chemical (3, 6) methods. Stability at 72° and 100°F. was examined over a period of one year.

Study 2. After Study 1 was in progress for several months, it was readily apparent that thiamine stability at 100°F. storage was poor. In order to ascertain the reliability of these data, canned bread was baked in the laboratory[7] under the most rigidly controlled conditions and again the stability of added thiamine was tested. Dried yeast[8] (debittered brewers') which had proved so satisfactory in the biscuit and cracker component was also added to canned bread from this batch to study the effect of the yeast on the stability of synthetic thiamine. Preliminary tests to determine the maximum amounts of debittered brewers' yeast which could be employed for enrichment without producing off-flavors showed the 3% level (flour basis) to be the most satisfactory. This study also presented the opportunity of determining baking losses since the doughs were available. Stability tests were made only at 100°F. storage temperature and only two cans of bread were assayed initially and after 3 and 6 months' storage because of the limited number of samples.

Study 3. This investigation was similar to that of Study 2 except that it was carried out on a test production run and the dried brewers'

[6] Winthrop high potency enrichment tablets were used, one tablet per 50 pounds of flour. The tablets contained 115 mg. of thiamine, 60.5 mg. of riboflavin, 725 mg. of niacin, and 625 mg. of iron per tablet.

[7] Canned bread was baked by the Cereal and Baked Products Branch. The dough was placed in No. 2½ cans, the cover clinched on, and the bread allowed to proof. Following the proofing stage, the dough was baked in a (450°F.) reel oven containing four trays (24 cans per tray), 96 cans being baked at one time. The cans were removed from the oven after 25–30 minutes, cooled to 190°F. (5 to 10 minutes at room temperature) and then sealed. The basic formula for canned bread in any one study was identical differing only in the type of enrichment made, i.e., substitution of white flour for whole wheat flour or addition of synthetic vitamins instead of dried yeast.

[8] Dead yeast cells have a deleterious effect on the gluten structure of dough (4). A method of treating the yeast to overcome this gluten-softening effect is essential before the yeast can be used successfully as a fortifying agent. The dry yeast used in Study 2 was not a "treated" yeast. Industry was contacted for the purposes of cooperating in the development of a yeast which would meet the above requirements.

yeast used was "treated" yeast.[9] Stability of thiamine, riboflavin, and niacin at 72° and 100°F. storage was tested at bimonthly intervals for one year.

Study 4. This extensive study was made on laboratory-baked bread to compare synthetic, whole wheat, and "treated" yeast enrichment on the basis of vitamin B complex stability, contribution of protein[10] and lesser known vitamin factors of the B complex, and palatability. The yeast was used at a 3% level. It was also possible to determine baking losses in each type of bread as well as fermentation losses.

Study 5. In this study other dried yeasts, primary grown and debittered brewers', were used in canned bread at a 1% level and in pan loaf bread at a 3% level to determine whether differences in the baking quality of the resulting bread varied with the different yeasts used. Pan loaves were scored for volume, color, and grain and photographs were taken. Canned breads were stored at 72° and 100°F. and tested for thiamine stability and palatability initially and at 3, 6, 9, and 12 months of storage.

TABLE I

PERCENTAGE LOSS OF B COMPLEX VITAMINS DURING FERMENTATION

Study 4	Thiamine	Riboflavin	Niacin
	μg./g.	μg./g.	μg./g.
Synthetic fortification			
After mixing	3.62	3.64	38.0
After proofing	3.46	3.59	39.3
Per cent loss	4.00	1.00	+ 2.0
Dried yeast fortification			
After mixing	4.26	3.87	41.8
After proofing	4.07	3.60	39.0
Per cent loss	4.00	4.00	7.0
Whole wheat fortification			
After mixing	3.88	3.14	39.9
After proofing	3.79	3.04	40.5
Per cent loss	2.00	3.00	+ 2.0
Average fermentation loss	3	3	1

Results

Vitamin Stability:

a. Fermentation Losses.

Fermentation losses included those incurred from the time that the ingredients were mixed into a dough until the time the dough was ready

[9] This yeast was developed by Haffenreffer Yeast Company, Jamaica Plains, Boston, Massachusetts.

[10] The three types of canned bread were evaluated for protein by Dr. P. R. Cannon, University of Chicago, on Committee on Food Research Project NU-3. A detailed report on this study is in press.

to be placed into the oven, a period of approximately three hours. It is seen from Table I that there were no significant losses in thiamine, riboflavin, or niacin in the three types of doughs studied.

TABLE II

PERCENTAGE LOSS OF B COMPLEX VITAMINS DURING BAKING OF CANNED BREAD

Study	Thiamine	Riboflavin	Niacin
	μg./g.	μg./g.	μg./g.
Study 2			
Synthetic fortification			
After proofing	9.44	2.62	30.2
After baking	8.12	2.43	31.2
Per cent loss	14.00	8.00	+ 3.0
Yeast fortification			
After proofing	6.65	5.95	55.9
After baking	5.41	5.22	54.7
Per cent loss	19.00	12.00	2.0
Study 4			
Synthetic fortification			
After proofing	3.46	3.59	39.3
After baking	2.72	3.63	41.1
Per cent loss	21.00	+ 1.00	+ 5.0
Dried yeast fortification			
After proofing	4.07	3.52	39.0
After baking	3.26	3.90	39.9
Per cent loss	20.00	+11.00	+ 2.0
Whole wheat fortification[1]			
After proofing	3.79	3.04	40.5
After baking	2.65	3.67	41.1
Per cent loss	30.00	+21.00	+ 1.0
Study 5			
Synthetic fortification			
After proofing	4.69	—	—
After baking	4.36	—	—
Per cent loss	7.00		
Yeast fortification (5 yeasts)			
After proofing	2.16–5.51	—	—
After baking	1.68–4.98	—	—
Per cent loss	10–20		
Average baking loss	16	+ 2	+ 3

[1] Not included in average since baking time was longer.

b. Baking Losses.

Baking losses were determined in three studies. Ten and one-half ounces of dough were placed in a No. 2½ can and white dough baked at 450°F. for 25–30 minutes and whole wheat dough for 40 minutes. Table II shows the percentage loss of thiamine, riboflavin, and niacin which occurred during the baking process.

It is seen that laboratory-baked bread lost approximately 15% of thiamine during the baking process. This figure is in good agreement with those found by others for this baking time (5, 9, 11, 13). The niacin and riboflavin content showed no significant changes on baking. There were no demonstrable differences in the baking losses of synthetically fortified and dried yeast-fortified bread. The thiamine loss in whole wheat bread, however, was somewhat higher due to the longer baking time (30% loss).

c. Storage Losses.

The stability data on canned bread fortified with various sources of the B complex indicated that canned bread was as good a carrier as

TABLE III

STABILITY OF B COMPLEX VITAMINS IN STORED CANNED BREAD, FORTIFIED WITH SYNTHETIC AND NATURAL SOURCES OF ENRICHMENT [1]

Source of fortification	Thiamine				Riboflavin				Niacin			
	72°F.		100°F.		72°F.		100°F.		72°F.		100°F.	
	6 mo.	12 mo.	6 mo.	12 mo.	6 mo.	12 mo.	6 mo.	12 mo.	6 mo.	12 mo.	6 mo.	12 mo.
	Per cent retention				Per cent retention				Per cent retention			
Natural												
Dried yeast	84	73	50	31	112	105	105	103	98	94	96	98
Whole wheat	82	75	46	30	88	96	109	100	95	96	93	95
Synthetic												
White	89	75	54	37	114	104	106	109	95	97	96	95
White and bleached raisin	66	60	44	31	100	100	100	100	86	100	90	100
Avg. retention	80	72	49	32	104	101	105	103	93	97	94	97

[1] The retentions in this table represent the average of all storage data taken from Studies 1–5.

ration biscuits for riboflavin and niacin but not for thiamine. Table III summarizes the retention of the thiamine, riboflavin, and niacin in canned bread. Retention of thiamine, riboflavin, and niacin followed the same pattern in the synthetic, yeast, and whole wheat breads. In general, the thiamine content decreased as in other moist carriers (approximately 50 and 20% loss after 6 months' storage at 100° and 72°F. respectively). The riboflavin and niacin content remained at the initial level at both storage temperatures throughout the storage period.

Upon further storage for an additional 6 months, the thiamine content continued to decline but at a slower rate than in the initial 6

months' storage period. The thiamine loss at the end of this time was approximately 70 and 30% at the 100° and 72°F. storage temperatures respectively. The thiamine loss in raisin bread was somewhat greater than in the other types of bread at 72°F. storage, and was in part attributed to the possible action of the bleaching agent used on the raisins.

This pattern of stability characterized by significant decreases in thiamine content with little if any decreases in the riboflavin and niacin content has been reported for other canned foods. The thiamine losses (6 months' storage at 100°F.) for canned meats were found to be higher than for canned bread ranging from 60 to 70% (7). In canned fruits and vegetables the thiamine losses were found to be somewhat lower than in canned bread averaging 40% (1, 10, and 12).

TABLE IV

ANALYTICAL VALUE FOR VARIOUS STORAGE STUDIES, CORRECTED TO 38% MOISTURE

	Storage time	Thiamine μg./g.		Riboflavin μg./g.		Niacin μg./g.	
		72°F.	100°F.	72°F.	100°F.	72°F.	100°F.
Study 1							
White bread + synthetic vitamins	Initial	1.95	—	2.27	—	29.4	—
	6 mos.	1.97	1.20	2.38	2.51	27.2	28.7
	12 mos.	1.65	0.83	2.23	2.78	27.3	29.3
Raisin bread + synthetic vitamins	Initial	1.08	—	1.69	—	24.7	—
	6 mos.	0.73	0.48	1.57	1.64	21.3	23.6
	12 mos.	0.65	0.33	1.62	1.77	25.0	24.9
Whole wheat bread	Initial	2.09	—	1.56	—	37.9	—
	6 mos.	1.59	0.87	1.42	1.47	37.2	36.7
	12 mos.	1.61	0.64	1.42	1.56	34.8	33.7
Study 2							
White bread + synthetic vitamins	Initial	8.61	—	2.56	—	33.1	—
	6 mos.	—	3.77	—	2.26	—	33.7
	12 mos.	—	—	—	—	—	—
Yeast fortification	Initial	5.75	—	5.54	—	58.1	—
	6 mos.	—	2.60	—	5.22	—	58.8
	12 mos.	—	—	—	—	—	—
Study 3							
White bread + synthetic vitamins	Initial	2.23	—	2.69	—	25.1	—
	6 mos.	1.65	1.20	3.05	3.00	24.8	24.5
	12 mos.	1.42	0.73	3.05	3.21	23.3	21.6
Yeast fortification	Initial	2.82	—	3.11	—	30.9	—
	6 mos.	2.27	1.54	3.40	3.16	34.0	31.9
	12 mos.	1.91	0.82	3.39	3.25	30.3	31.4

TABLE IV—*Continued*

	Storage time	Thiamine µg./g.		Riboflavin µg./g.		Niacin µg./g.	
		72°F.	100°F.	72°F.	100°F.	72°F.	100°F.
Study 4							
White bread + synthetic vitamins	Initial	2.81	—	3.93	—	41.9	—
	6 mos.	2.64	1.62	4.89	4.64	39.2	38.3
	12 mos.	2.36	1.14	3.92	3.79	41.7	39.8
Yeast fortification	Initial	3.20	—	3.72	—	42.5	—
	6 mos.	2.80	1.73	4.32	4.47	36.2	36.2
	12 mos.	2.50	1.08	4.00	3.82	40.7	39.8
Whole wheat	Initial	2.57	—	3.29	—	40.9	—
	6 mos.	2.27	1.29	3.03	4.08	37.9	37.3
	12 mos.	1.95	0.75	3.25	3.28	41.5	40.0
Study 5							
White bread + synthetic vitamins	Initial	3.89	—				
	6 mos.	3.42	1.77				
	12 mos.	3.07	1.35				
Yeast fortification A.	Initial	4.48	—				
	6 mos.	3.92	2.05				
	12 mos.	3.79	1.40				
B.	Initial	5.22	—				
	6 mos.	4.15	2.49				
	12 mos.	3.86	1.23				
C.	Initial	4.21	—				
	6 mos.	3.39	1.91				
	12 mos.	3.41	1.24				
D.	Initial	3.89	—				
	6 mos.	3.27	2.19				
	12 mos.	3.16	1.55				
E.	Initial	3.06	—				
	6 mos.	—	1.50				
	12 mos.	—	—				

Protein Quality. The protein of the three types of canned bread in study four was evaluated by feeding the dried bread at a 9% protein level to protein depleted adult rats for 14 days and determining the resulting weight recovery at the end of that time (2). The weight gains for rats fed these breads showed that both the whole wheat and the yeast enriched bread was approximately 30% superior in protein quality to the white synthetically enriched bread.

Evaluation for Lesser Known Vitamin Factors.[11] The presence of the lesser known vitamin factors in the canned breads was determined

[11] Evaluation of the canned breads for the lesser known vitamin factors by their growth-promoting value for rats was conducted by Capt. C. French of the Medical Nutrition Laboratory, Surgeon General's Office.

by comparative growth rates of rats fed on equicaloric diets containing 80% bread and 20% basal supplement. The basal supplement contributed adequate amounts of protein, fat, mineral, thiamine, riboflavin, niacin, choline, tocopherol, and vitamins A and D. Growth rate obtained from the three bread diets when compared to a control group receiving Purina Laboratory Chow indicated that no significant rat growth deficiency was involved in any of the three bread diets.

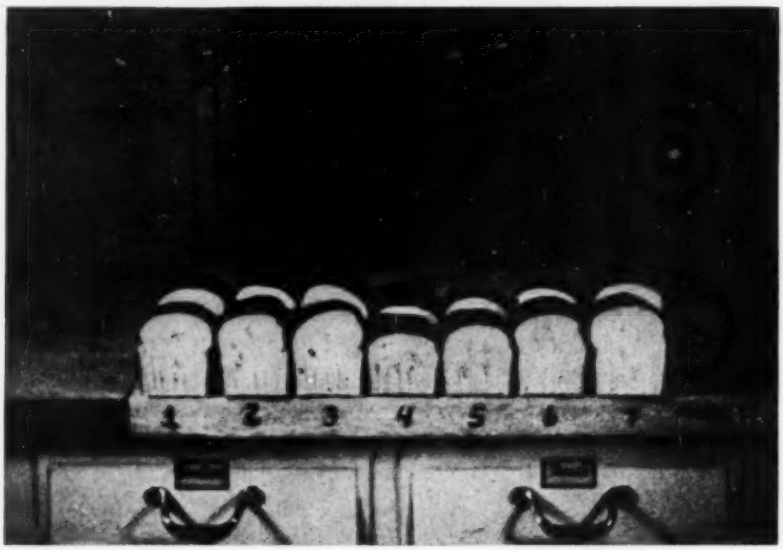

Fig. 1. Effect of 3% yeast addition (flour basis) on bread score.

Bread 1. Primary grown yeast
2. Debittered brewers' yeast (treated to overcome gluten softening effects)
3. Primary grown yeast
4. Debittered brewers' yeast
5. Primary grown yeast
6. Primary grown yeast
7. Control made with enriched flour

Palatability.[12] Examinations were made initially and during storage on the bread of Studies 4 and 6. Initially, the 3% yeast bread was judged to be not equal to the synthetically enriched bread in color, grain, and eating quality, and was described as having a bitter, undesirable after taste. The 1% yeast breads were rated as equally as acceptable as the synthetically enriched breads. There were no yeasty or bitter flavors and the color of the grain was lighter than at the 3% level although variations among the yeasts in degree of color produced were evident.

The effects of storage on palatability were more pronounced at the higher storage temperatures. All breads declined in acceptability on

[12] Palatability studies, by taste panels, were conducted by the Food Acceptance Research Branch, QM Food and Container Institute for the Armed Forces.

storage. The 3% yeast bread declined to a greater degree than either the synthetically enriched bread or the 1% yeast bread, the yeasty flavor becoming more apparent and undesirable on prolonged storage. There was no appreciable difference in acceptability of the stored 1% yeast breads and the synthetically enriched breads, thus indicating that the addition of 3% yeast is too high.

Baking Quality. This was determined from the bread score which was made up of individual scores on volume, crumb color, grain, texture, and flavor. The resulting pan loaves made from the six yeasts used at a 3% level (flour basis) are shown in Fig. 1. The amount of gluten softening effect produced by the various yeasts is reflected in the loaf volume. The deleterious effect of the dead yeast cells is especially evident in breads 4 and 5. Bread 2 was made with the treated yeast used in Study 4. The crumb color of all the yeast breads was darker than for synthetically fortified bread (bread 7), breads 4 and 5 having the darkest crumb color. Grain and texture was also scored lowest for breads 4 and 5. Taste tests indicated that the synthetically fortified sample was preferred over the yeast fortified samples with little or no preference for the different yeast breads.

Summary

1. Fortified canned bread was investigated as a possible carrier of the B complex vitamins. Comparisons of synthetic, whole wheat, and yeast enrichment were made for relative vitamin stability and palatability. Fermentation and baking losses were also investigated.

2. There were no significant losses of the B complex vitamins through the fermentation and proofing step. During baking the thiamine content of canned bread was decreased by approximately 15%; the riboflavin and niacin content remained unchanged.

3. During storage at 72° and 100°F. for 6 months the thiamine retention was approximately 80 and 50%, respectively, regardless of the source of enrichment. The use of bleached raisins in white bread increased the losses. Riboflavin and niacin were retained from 90 to 100% for all sources of enrichment at both storage temperatures.

4. There were no deficiencies of the lesser known vitamins in the three types of bread tested (whole wheat, synthetically fortified, and yeast fortified bread) as judged from their growth-promoting values.

5. Yeast and whole wheat bread had better protein value than synthetically fortified bread.

6. A 1% level of yeast fortification was more satisfactory than a 3% level (flour basis), the former producing a bread more nearly equal in color, grain, texture, and taste to bread fortified with synthetic vitamins.

Acknowledgments

The authors wish to express their appreciation to Mr. T. Soloski and Mr. J. Cryns of the Cereal and Baked Products Branch for the preparation of the canned bread and for the technical scoring of these breads and to Dr. F. W. Dove and other members of the Food Acceptance Research Branch for conducting and evaluating the acceptability tests.

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INFLUENCE OF AUTOCLAVING DRY PEAS ON SOME PROPERTIES OF THE PROTEINS [1]

ROBERT JOHN EVANS [2] and J. L. ST. JOHN [3]

ABSTRACT

The influence of autoclaving dry peas on the peptization and digestibility of the protein has been determined. Autoclaving finely ground dry Alaska peas decreased the protein dispersion by water at pH 1.8 and 10 and by a 1.0 *N* sodium chloride solution in the pH range 6 to 12. The per cent protein soluble in water and in saline solution was decreased. Mild autoclaving (110°C. for 30 minutes) increased the per cent of alkali-soluble protein, but more drastic autoclaving (130°C. for 60 minutes) again decreased it. The percentage of nitrogenous compounds peptized by different salt solutions was decreased by autoclaving the peas, particularly when drastic autoclaving was employed.

The liberation of amino groups by *in vitro* enzymic digestion of dry peas was decreased by autoclaving as was the liberation of methionine and lysine by *in vitro* trypsin and erepsin digestion. Autoclaving at 130°C. for 60 minutes caused a destruction of 24% of the cystine, 25% of the lysine, and none of the methionine in dry peas as determined by microbiological assay.

The effect of autoclaving soybean oil meal on the proteins has been the subject of considerable study in this laboratory since the observation of Evans and St. John (7) that the poor nutritive values of some commercial soybean oil meals was caused by overcooking. It had previously been shown by other investigators that cooked soybean oil meals were better nutritionally than raw meals (8, 9). Subsequent work has been conducted to cast more light on the nature of the effect of heat-treatment on soybean oil meal proteins.

Soybean oil meal proteins have been known for some years to behave differently from most other proteins with regard to the effect of heat on nutritive value (12). It was believed desirable to study the influence of autoclaving on other proteins than those of soybean oil meal. Eggs and dry peas were chosen. The data on eggs are presented elsewhere (14). Although peas are a legume, as are soybeans, the nutritive value of dry peas is not improved by cooking (17). Because of this difference between dry peas and soybeans and because

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of the economic importance of dry peas in eastern Washington, they were chosen for the present investigation.

Materials and Methods

A sample of finely ground dry Alaska peas[4] was divided into three portions. One portion was not autoclaved, one was autoclaved for 30 minutes at 110°C., and the other was autoclaved for 60 minutes at 130°C.

Peptization studies on the three samples were carried out at different pH levels using hydrochloric acid and sodium hydroxide to adjust the pH as described by Evans, Henry, and St. John (4). A similar investigation was made by peptizing with 1.0 *N* sodium chloride solution at different pH levels (4) and six different salts at the normal pH of the salt solutions.

To determine the influence of autoclaving on protein solubility, water-soluble nitrogen, nitrogen soluble in saline solution, alcohol-soluble nitrogen, alkali-soluble nitrogen, and residual nitrogen, determinations were made on each of the three samples by the method of Lund and Sandstrom (11).

In vitro enzyme digestions of the three pea samples were carried out as described by Evans (3). Pepsin, trypsin, trypsin followed by erepsin, and pepsin followed by trypsin followed by erepsin were the enzymes used in this study.

To determine methionine, cystine, and lysine in the dry peas two methods of hydrolysis were used to liberate the amino acids. In the first, 2.0-g. samples of dry peas were hydrolyzed by autoclaving at 15 pounds pressure for 8 hours with 10 ml. of 10% hydrochloric acid in a sealed tube. For the second method 2.0-g. samples of the dry peas were digested with the enzymes trypsin and erepsin as described by Evans (3). Amino nitrogen was determined by the Van Slyke method (16), methionine with *Lactobacillus arabinosus* (15), lysine with *Streptococcus faecalis* (1), and cystine in the acid digest with *L. arabinosus* (15), and in the enzyme digest with *Leuconostoc mesenteroides* (15) using entirely synthetic media.

Results and Discussion

Protein Solubility in Various Reagents. Evans and St. John (7) observed that a very pronounced change in the solubility of the proteins in raw soybean oil meal resulted from autoclaving. There was a decrease in the proteins soluble in saline solution, an increase followed by a decrease in the alkali-soluble proteins, and an increase in

[4] The dry Alaska peas were kindly furnished by E. A. Dumas of the Klemgard Pea Processing Company of Pullman, Washington.

TABLE I

INFLUENCE OF AUTOCLAVING DRY PEAS ON PROTEIN SOLUBILITY IN VARIOUS REAGENTS

Protein fraction	Not autoclaved	Autoclaved at 110°C. for 30 min.	Autoclaved at 130°C. for 60 min.
Water soluble (%)	59.1	25.3	21.8
Soluble in saline solution (%)	20.6	16.3	3.2
Alcohol soluble (%)	1.6	2.9	2.8
Alkali soluble (%)	5.2	29.4	6.7
Residual (%)	14.3	27.3	66.3

the residual protein. Similar data for dry peas are presented in Table I. As with soybean oil meal, autoclaving caused a decrease in the protein fractions soluble in water and in saline solution and an increase in the residual protein fraction. Autoclaving at 110°C. for 30 minutes increased the amount of protein in the alkali-soluble fraction. Autoclaving at 130°C. for 60 minutes resulted in a low alkali-soluble protein fraction again. These results are all in agreement with those previously reported for soybean oil meal.

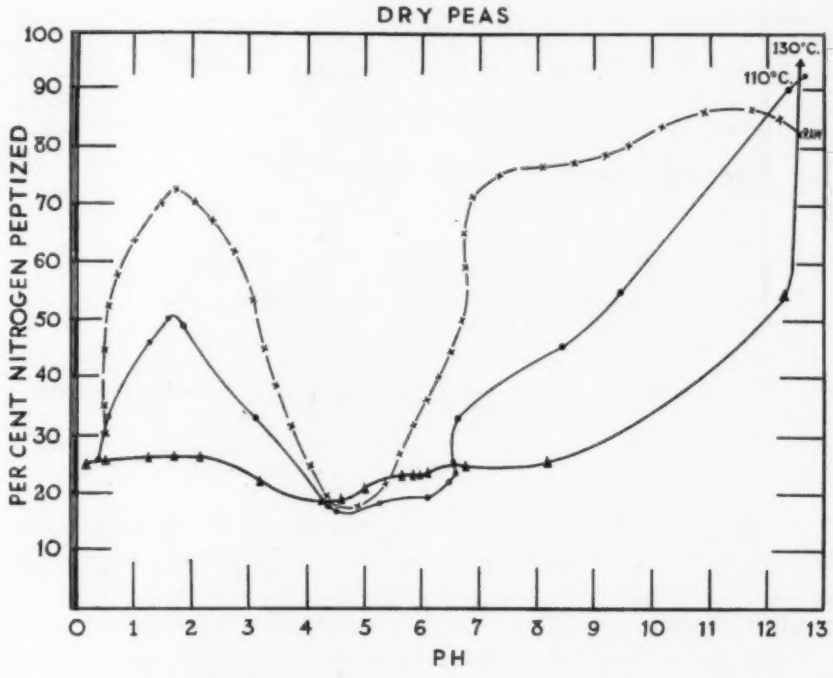

Fig. 1. The influence of autoclaving dry peas on the peptization of the nitrogenous constituents by solutions of different pH values.

Raw peas X——X
Peas autoclaved for 30 min. at 110°C. 0——0
Peas autoclaved for 60 min. at 130°C. △——△

Although no feeding studies were conducted, the data presented here for the alkali-soluble proteins taken with the work of Woods, Beeson, and Bolin (17) indicate that there would be no relationship between the per cent of protein in the alkali-soluble fraction and the protein nutritive value of dry peas such as was observed with soybean oil meal by Evans, McGinnis, and St. John (6). Woods, Beeson, and Bolin (17) observed that an increasingly harmful effect on the nutritive value of dry peas for rats resulted from increased heating as contrasted to the improvement which first occurs in soybean oil meal.

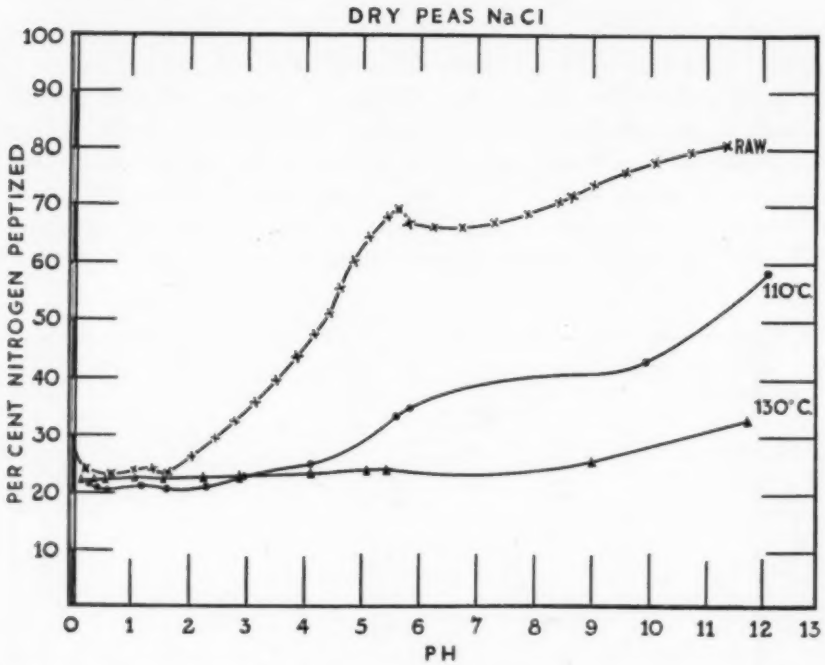

Fig. 2. The influence of autoclaving dry peas on the peptization of the nitrogenous constituents by 1.0 *N* sodium chloride solution of different pH values.

Raw peas X——X
Peas autoclaved for 30 min. at 110°C. 0——0
Peas autoclaved for 60 min. at 130°C. △——△

Peptization of Protein at Different pH Levels. To investigate further the influence of autoclaving on protein dispersion, peptization curves were obtained using as peptizing agents water or 1.0 *N* sodium chloride solution, the pH of which was adjusted to different values with sodium hydroxide or hydrochloric acid. The results are presented in Figs. 1 and 2. Evans, Henry, and St. John (4) earlier obtained a clear-cut peptization curve with dry peas using hydrochloric acid and sodium hydroxide to adjust the pH of the suspensions. They also

obtained an interesting curve using a 1.0 *N* sodium chloride solution. It was believed desirable to determine the influence of autoclaving dry peas on the shape of these curves. Points of maximum dispersion on the hydrochloric acid-sodium hydroxide curve were at pH 1.8 and 12.3. Minimum dispersion occurred at pH 4.6. Peptization at pH 1.8 was decreased by autoclaving the peas at 110°C. for 30 minutes. It was further decreased by autoclaving at 130°C. for 60 minutes until it was little greater than that occurring at the point of minimum peptization.

At the point of minimum peptization there was little difference in the per cent of protein peptized from the raw and the different heat-treated peas. Since the 18% of nitrogenous compounds soluble at pH 4.6 was not decreased by autoclaving, this fraction is either non-protein in nature, or belongs to some protein group that is not denatured by the heat treatment here used.

TABLE II

INFLUENCE OF AUTOCLAVING DRY PEAS ON PROTEIN SOLUBILITY OF THE PROTEINS IN 1.0 N SALT SOLUTIONS

Salt	Not autoclaved		Autoclaved at 110°C. for 30 min.		Autoclaved at 130°C. for 60 min.	
	% *N*		% *N*		% *N*	
	pH	Peptized	pH	Peptized	pH	Peptized
NaCl	5.7	66.7	5.8	31.8	5.3	24.0
Na_2SO_4	5.8	69.4	5.9	32.2	5.4	23.6
$CaCl_2$	5.0	61.1	5.1	34.1	4.8	23.8
$AlCl_3$	3.3	35.3	3.6	22.0	3.6	20.4
Na_3PO_4	11.1	69.0	11.4	51.6	11.4	30.0
Li_2SO_4	5.5	69.0	5.6	35.1	5.2	25.4

Autoclaving at 110°C. decreased the amount of protein peptized at pH 7.0, which would be considered a relatively mild treatment, to almost as low a value as for the peas autoclaved at 130°C., which was little greater than at the point of minimum dispersion.

At pH 12.5 the nitrogenous substances from all of the pea samples were over 80% peptized. This might have resulted from a breakdown of the protein by the strong alkali.

Nelson (14) conducted a similar study with eggs. Results with egg yolk proteins were in most respects similar to those with dry peas; boiling for 10 minutes reduced the solubility of the nitrogen compounds to about 4% at pH values below 9. Boiling decreased the amount of nitrogenous compounds in egg yolk, which were soluble at the point of minimum peptization, in contrast to the data with peas. However,

the dispersion characteristics of egg yolk proteins and peas are influenced similarly by heat. The solubility of egg albumen was decreased from above 90% in the range pH 1–12 for the raw albumen to 10% of the albumen of the eggs boiled for 30 minutes.

Peptization of Proteins by Different Salt Solutions. Autoclaving dry peas decreased the dispersion of the proteins in 1.0 *N* solutions of six different salts. Autoclaving at 130°C. gave a greater decrease than at 110°C. (Table II). The pH values of the suspensions were generally slightly lower for peas autoclaved at 130°C. than for raw peas.

Liberation of Amino Nitrogen by Proteolytic Enzymes. The results of enzyme digestion studies are presented in Table III. In all cases, autoclaving decreased the extent of digestion, the decrease being more pronounced the higher the temperature and the longer the time of autoclaving. Evans (3) observed an increased liberation of amino groups by trypsin or trypsin and erepsin after autoclaving raw soybean oil meal for 30 minutes at 110°C., while a decreased liberation followed

TABLE III

INFLUENCE OF AUTOCLAVING DRY PEAS ON LIBERATION OF AMINO NITROGEN BY PROTEOLYTIC ENZYMES
(PER CENT OF TOTAL NITROGEN LIBERATED)

Enzymes used	Not autoclaved	Autoclaved at 110°C. for 30 min.	Autoclaved at 130°C. for 60 min.
Pepsin	17	16	11
Trypsin	53	45	31
Trypsin-erepsin	46	40	34
Pepsin-trypsin-erepsin	38	39	35

the 130°C. for 60 minutes autoclaving treatment. The peptide bonds of raw peas were hydrolyzed by trypsin or trypsin and erepsin to a greater extent than those of peas autoclaved for 30 minutes at 130°C. Thus, the proteins of soybean oil meal are rendered more digestible and those of peas less digestible by the mild autoclaving treatment. Since the beneficial results with soybeans are attributed to a destruction of the trypsin inhibitor present, it may be inferred that a trypsin inhibitor is not present in dry peas; actually none was found in peas by Borchers *et al.* (2). The differences in digestibility might well account for the differences in nutritive value between soybeans and peas which result from autoclaving. Evans, McGinnis, and St. John (6) observed a close relationship between *in vitro* trypsin and erepsin digestion and chick digestion of soybean oil meal proteins.

Nelson (14) studied the influence of boiling on the *in vitro* enzymic digestion of the proteins of egg yolk and egg albumen. Egg albumen behaved in much the same manner as soybean oil meal, since boiling

for 30 minutes increased the extent of hydrolysis by trypsin or trypsin and erepsin. Egg whites contain a trypsin inhibitor, which has been identified with ovomucoid (10), which accounts for the similarity of behavior between soybeans and egg whites. On the other hand, egg yolk proteins behaved more like those of dry peas because the 30-minute boiling decreased trypsin and erepsin digestion.

Methionine, Cystine, and Lysine Liberation by Acid and by Enzyme Digestion. McGinnis and Evans (13) observed that soybean oil meal which had been autoclaved at 130°C. for 60 minutes gave normal growth with chicks when the chick diet was supplemented with 0.5% each of methionine, cystine, and lysine, but not otherwise. They,

TABLE IV

INFLUENCE OF AUTOCLAVING DRY PEAS ON LIBERATION OF METHIONINE, CYSTINE, AND LYSINE BY ACID AND BY *in vitro* TRYPSIN AND EREPSIN DIGESTION

Determination	Method of hydrolysis	Not autoclaved		Autoclaved at 110°C. for 30 min.		Autoclaved at 130°C. for 60 min.	
		In peas	Enzyme acid	In peas	Enzyme acid	In peas	Enzyme acid
		%[2]	%	%[2]	%	%[2]	%
Amino nitrogen	Acid	2.65		2.65		2.56	
	Enzyme	1.84	69	1.56	59	1.38	54
Methionine	Acid	0.31		0.29		0.36	
	Enzyme	0.12	39	0.11	38	0.08	22
Cystine	Acid	0.16		0.17		0.13	
	Enzyme[1]	(0.53)	(331)	(0.54)	(318)	(0.43)	(331)
Lysine	Acid	1.56		1.60		1.20	
	Enzyme	0.99	63	0.75	47	0.53	44

[1] Partial digests of soybean oil meal give a stimulating cystine action for the organisms *Lactobacillus arabinosus* and *Leuconostoc mesenteroides* on the synthetic type of media used which was greater than could be accounted for if all the sulfur in the soybean oil meal were cystine. The value for cystine is considerably higher for the enzyme digest than for acid ones, and the enzyme values are given here for comparative purposes only.

[2] Per cent on a basis of total weight of the dry peas.

therefore, concluded that the deficiencies of overcooked soybean oil meal could be accounted for by inactivation or methionine, cystine, and lysine. For that reason the influence of autoclaving on the amino nitrogen, methionine, cystine, and lysine content of dry peas, both after acid and enzyme hydrolysis, was determined with the results shown in Table IV. The value after acid hydrolysis supposedly represents total, and that after *in vitro* trypsin and erepsin digestion, available amino acids.

Although no loss of total methionine occurred, losses amounting to 24% of the cystine and 25% of the lysine were caused by the autoclaving the peas for 60 minutes at 130°C. Evans and McGinnis (5)

observed similar results from the more drastic autoclaving of soybean oil meal. The slight decrease in amino nitrogen resulting from the more drastic autoclaving was in all probability caused by the destruction of cystine and lysine.

There was a progressive decrease in the per cent of amino nitrogen, methionine, and lysine liberated from dry peas by *in vitro* trypsin and erepsin digestion after increasingly drastic autoclaving treatment. The results from more drastic autoclaving are similar to observations of Evans and McGinnis (5) with soybean oil meal. Although a similar decrease did not occur in cystine availability, the data are only suggestive, since the values obtained after enzyme digestion were over three times as high as the total cystine determined on the acid digest. Both *Leuconostoc mesenteroides* and *Lactobacillus arabinosus* give high results for cystine in partially hydrolyzed soybean oil meal or dry peas on the type of media used. Apparently the partially hydrolyzed meals stimulate these organisms on such synthetic media. Prolonged acid hydrolysis often results in considerable losses of cystine, so that the total cystine values may be low. At least the results are suggestive, even though not conclusive, that autoclaving at 130°C. did not reduce the percentage of cystine present that was liberated by *in vitro* trypsin and erepsin digestion. Similar results have been obtained with *in vitro* pepsin, trypsin, and erepsin digestion of soybean oil meal. No increased enzymic liberation of methionine, cystine, and lysine resulting from the mild autoclaving of dry peas was observed such as occurred with raw soybean oil meal (5).

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EVALUATION OF THE BAKING PROPERTIES OF ROLLER PROCESS NONFAT DRY MILK SOLIDS BY A FARINOGRAPH PROCEDURE [1]

CHARLES HOFFMAN, T. R. SCHWEITZER, E. K. SPOTTS, and GASTON DALBY[2]

ABSTRACT

A farinograph procedure for evaluating the quality of nonfat dry milk solids for breadmaking is described. The procedure involves a determination of the absorption of a mixture of equal parts of spring wheat flour and nonfat milk solids after 10 to 14 minutes of mixing to allow time for any softening action of the milk solids on the gluten to take place. From a knowledge of the absorption of the flour itself, the absorption due to the milk solids can be computed. The absorption as measured by this procedure is a summation of two factors: the actual physical absorption of water by the dry milk plus a measure of its softening action. Nonfat milk solids of satisfactory baking quality give absorption values of 70% or higher, while solids of low quality give values of 65% or lower.

Ever since the introduction of nonfat dry milk solids into the baking industry the evaluation of their baking properties has presented difficulties. A sample with poor baking properties in commercial production may, and in most cases does, give reasonably good laboratory baking results. To overcome this anomaly, it is customary in experimental baking practice to increase the percentage of milk based on the flour to as high as 12%. Extra punishment of the dough such as repeated molding has also been tried. No definitive simulation of shop conditions which will show up a milk of intermediate value has yet come into general use.

[1] Manuscript received April 12, 1948.
[2] Ward Baking Company, New York, N. Y. With the technical assistance of Robert G. Scherf.

Many laboratories have measured the viscosities of mixtures of flour, milk, water, and some correlation with baking properties of the milk has been obtained. Since the introduction of dough testing apparatus many efforts have been made to measure a softening effect of milk on flour. Usually 6% to 10% dry milk based on the flour is used. Skovholt and Bailey (6) obtained a positive correlation between dough plasticity and baking properties of dry milks. The practical difficulty in these earlier studies has been the lack of a clear-cut evaluation of nonfat milk solids of intermediate baking properties.

Under shop conditions nonfat milk solids of poor baking quality result in soft, slack doughs, prolonged proofing time, and poor oven spring. The bread has small loaf volume and poor crumb and texture. Hence all known facts suggest a definite correlation between "baking quality" of milk solids and dough consistency.

The presence of increasing quantities of milk should magnify this effect. To test this theory, preliminary Brabender Farinograph studies were made with two milks, one of which gave good commercial results while the other gave very poor commercial results. The proportions of flour and nonfat milk solids in the mix were varied from one extreme to the other in 10% increments and the most pronounced result was obtained with a 50–50 mixture of flour and milk solids.

Method

The absorption of a spring wheat flour is determined by titrating a 300-g. sample ("as is" basis) with water until a maximum dough development of 500 Brabender Units is obtained. Place 150 g. of the flour, 150 g. of the nonfat milk solids in the farinograph mixer, and add the amount of water computed as follows: Let F be the absorption of the flour in percentage based on the original titration of the flour. Then the percentage water to be added to the mixture will be: $\frac{F + 65}{2}$. (Thus an absorption of 65% is arbitrarily assigned to the milk.) An average sample of nonfat milk solids when mixed with flour will reach the 500 line immediately, but within 30 seconds or so the mixture will soften. After 10 to 14 minutes mixing the pointer will again reach the 500 line. Then add more water in 1.5 ml. portions, to hold the pointer on the 500 line during further mixing until maximum absorption is measured. Let X be the final absorption of the mixture; the absorption of the nonfat milk solids is $2\left(X - \frac{F}{2}\right)$. Record two factors: 1. Development period (the time required in minutes for the mixture to reach a consistency of 500 with the original addition of water). 2. Final absorption of milk.

Results and Discussion

Using one sample of nonfat milk solids, tests with strong spring patent and weak Southwestern patent flours have given essentially the same results. This test has been repeated with milks of various degrees of quality. The type of flour used in the test is not, therefore, a critical factor. A sample of commercially ground roller process nonfat milk solids was screened into three particle size fractions. Farinograph tests on each fraction were similar. This procedure was also repeated with milks of various degrees of quality. Particle size had no effect.

Using the above method with nonfat milk solids of poor, intermediate, and good baking quality and a flour of 63.0% absorption, the curves shown in Fig. 1 were obtained. The absorption of the poor milk was less than 65%, since the consistency of the mixture never reached 500 Brabender Units. The development period is recorded

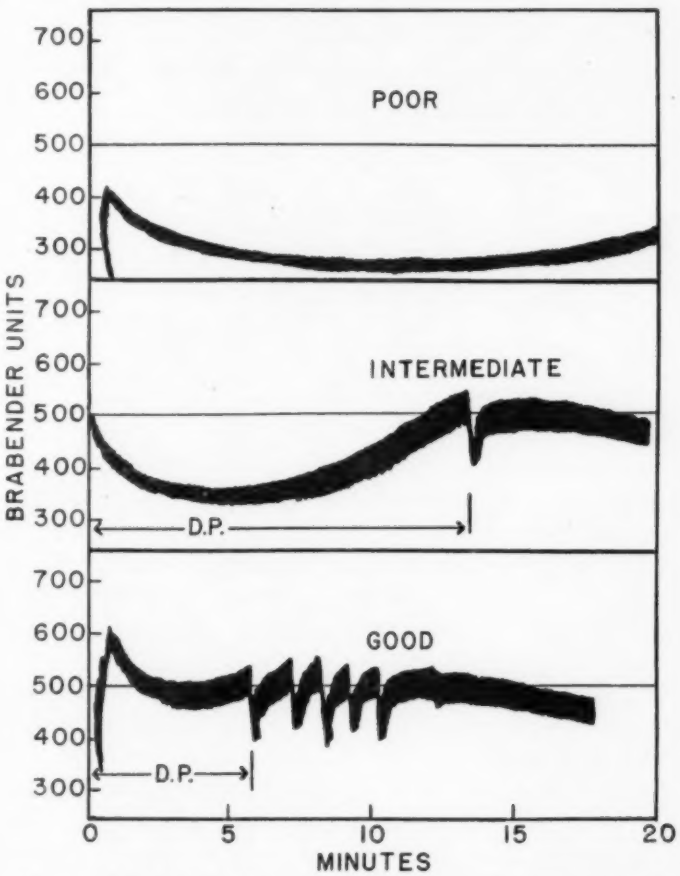

Fig. 1. Farinograph charts for equal mixtures of flour and poor, intermediate, and good quality nonfat milk solids, respectively.

as "never reached." The sample of intermediate quality gave a development period of 12.5 minutes and a calculated final absorption of 67%. The nonfat milk solids of good baking properties had a final absorption of 76.5%. The development period was 6 minutes.

The absorption figure obtained by this determination is not entirely a measure of the actual absorption of the milk itself, but a summation of the softening action of the milk on the flour and the actual physical absorption of the milk. This figure is of paramount interest to the baker.

The development time, in theory, depends upon an equilibrium. On mixing the flour, the gluten undergoes development, the rate of which is influenced by the properties of the nonfat milk solids and is, therefore, a useful index of the quality of the milk.

A sample of nonfat milk solids of poor baking quality was tested in the usual manner and the chart made. Then 150 g. of flour were

TABLE I

FARINOGRAPH ABSORPTION VALUES OF EQUAL MIXTURES OF FLOUR AND NONFAT MILK SOLIDS OF VARYING QUALITY ADDED BEFORE AND AFTER GLUTEN DEVELOPMENT

Quality	Absorption[1] Milk solids added	
	Before gluten development %	After gluten development %
Poor[2]	Less than 65	67
Intermediate[2]	67	72
Good[2]	76.5	80

[1] Absorption figures are those calculated for the milk solids, and are not the absorption values of the mixture of flour and milk solids.
[2] Same milk samples from which the curves in Fig. 1 were obtained.

placed in the farinograph, the amount of water needed for the 150 g. of flour added, and the dough mixed for the normal time required for the particular flour to reach its mixing peak. (The chart did not read a consistency of 500 since insufficient total dough was in the mixer to read normally.) The machine was stopped and 150 g. of the milk solids and 97.5 ml. of water added and the mixing was resumed (97.5 ml. of water is equal to 65% absorption on 150 g. of milk solids). Since the gluten had an opportunity to develop by itself before the milk solids were added, the effect of the milk on gluten development was not nearly so marked.

As shown in Table I there is considerable improvement in the absorption value of a poor quality milk when it is added to the flour after the gluten has developed. The increase in absorption of an intermediate quality milk is not so great, and in the case of the good quality

milk is still proportionately less. These data lend some support to the theory that a milk poor in baking quality affects the dough by interference with gluten development.

It has been known for many years that heat treatment of milk was necessary for good baking properties. Geddes and his associates (1), Harland and Ashworth (2), and, earlier, Skovholt and Bailey (5) have shown that the adverse effect of a poor milk is due to its content of undenatured serum protein which depends upon the time and temperature of preheat treatment. Measurement of the percentage of undenatured serum protein present in a sample of nonfat milk solids should, therefore, serve as a useful index of its quality for baking. In fact, Ashworth (2) has published a technique for this purpose. Geddes and associates (1) have suggested that the Rowland procedure (3, 4) for determining the nitrogen distribution in milk might prove valuable for the evaluation of milk for bakery purposes.

Six milks of various degrees of baking quality have been tested by the farinograph procedure and by the methods proposed by Geddes (1)

TABLE II

COMPARISON OF GEDDES,[1] ASHWORTH, AND FARINOGRAPH PROCEDURES WITH QUALITY

Sample number	Undenatured serum protein N Per cent of total N	Rating
	GEDDES PROCEDURE[1]	
1	5.37	Good
2	5.50	Good
3	6.63	Good
4	10.63	Poor
5	8.14	Good
6	9.57	Poor
	ASHWORTH PROCEDURE Whey protein N (mg. per g.)	
1	0.5	Good
2	0.0	Good
3	0.8	Good
4	1.1	Good
5	1.7	Poor
6	1.4	Good
	FARINOGRAPH PROCEDURE	
	Development period	Absorption %	Rating
1	8½ minutes	71½	Excellent
2	7½ minutes	76	Excellent
3	Never reached	Less than 65	Poor
4	Never reached	Less than 65	Poor
5	0 minutes	82	Excellent
6	Never reached	Less than 65	Poor

[1] The Geddes application of the Rowland procedure for the evaluation of milk solids for bakery purposes.

and by Harland and Ashworth (2). The results are shown in Table II. Commercial results check best with the farinograph procedure ratings.

The Geddes adaptation of the Rowland procedure and the Ashworth technique may be of great value in the study of milk processing as well as to the baker. The farinograph procedure, however, enables the laboratory technician to determine the effect of nonfat milk solids on gluten development and on absorption. A comparison of commercial results (based on hundreds of tests) with the Brabender Farinograph procedure permits the following tentative ratings as shown in Table III.

TABLE III

TENTATIVE QUALITY RATINGS OF MILKS FROM RESULTS OF FARINOGRAPH TESTS

Development period	Absorption %	Rating
Under 10 minutes	Over 70	Excellent
10 to 12 minutes	Over 70	Good
12 to 16 minutes	Over 70	Fairly good
12 to 16 minutes	67–70	Fair
Over 16 minutes	Under 67	Poor

Although comparatively few tests with the farinograph procedure have been carried out on nonfat milk solids made by the spray process, the results indicate that the test holds equally as well as for roller process nonfat milk solids. The only modification necessary in the procedure is the use of an original 40% absorption on the milk rather than the 65% absorption figure taken for roller process milks. Since no commercial testing has been done with spray milks to correlate results with the farinograph procedure, the ratings given in Table III do not apply to spray process nonfat milk solids.

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THE DISPOSITION OF LIPASE AND LIPOXIDASE IN BAKING AND THE EFFECT OF THEIR REACTION PRODUCTS ON CONSUMER ACCEPTABILITY [1]

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ABSTRACT

Data are presented on the lipase activity of certain wheat fractions and bread constituents. Lipoxidase assay values for several wheat products, enzyme preparations, and baking ingredients also are listed.

The lipase and lipoxidase activity of normal baking ingredients, as well as that present in such enzymatically active adjuncts as soybean meal and wheat germ, is completely inactivated during the baking process. These enzymes are thus nonfunctional during the storage of baked goods, and any hydrolytic or oxidative rancidity occurring in such products must be due to chemical hydrolysis and autoxidation, respectively.

The products of lipolytic action have a detrimental effect on loaf characteristics. Unsaturated fatty acids are more detrimental to loaf characteristics than are saturated fatty acids; however, both types exert a marked detrimental effect on consumer acceptability in concentrations as low as 0.5 of 1%. Oxidized shortenings do not affect baked loaf characteristics appreciably but they do affect consumer taste reactions adversely. The release of fatty acids and the oxidation of lipid material by enzyme action during storage of flour, however, constitute an important economic problem.

Available data indicate that both hydrolytic and oxidative rancidity occur in flour and other wheat products as the result of lipase and lipoxidase action. In baked products rancidity is believed to be due mainly to autoxidation, although no experimental proof of this is available in the literature.

The distribution of lipase in wheat has been investigated by Sullivan and Howe (15), Pett (10), and Engel (5). In addition to the known lipase activity of flour and other wheat fractions, various baking adjuncts are also known to possess lipolytic activity. Van Laer (18) reported the presence of lipase in malt extract, and Gorback and Günter (6) observed the same enzyme in yeast. Certain possible baking adjuncts including wheat germ and soybean flour also contain large amounts of these enzymes.

The literature concerning the relation of esterases in milling and baking has been reviewed recently by Sullivan (14). If present in sufficient quantities the lipolytic enzymes may have detrimental effects

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on the bread product due to the free fatty acids released from either the added shortening or the natural lipid material of flour prior to baking. Barton-Wright (2) found that doughs made from flour stored at high moisture levels and relatively high temperatures lacked extensibility. The volume, flavor, and taste of the bread also were inferior. Sullivan, Near, and Foley (16) and Barton-Wright (2) reported that small amounts of unsaturated fatty acids such as oleic, linoleic, and linolenic acid did not injure the breadmaking characteristics to the extent that might be expected from the deleterious effect on gluten. Sullivan *et al.* (16) showed that the damaging action on breadmaking occurred only when the unsaturated acids became rancid or were oxidized either by air or by the lipoxidase enzymes in flour. The oxidized unsaturated fatty acids produced a dough which was "dead" and a bread with poor volume, flavor, and taste.

The presence of the enzyme lipoxidase has been demonstrated in wheat germ although the quantity is only 2.5% of that found in soybean meal (Sumner, 17). No data are available concerning the lipoxidase content of patent flour. This enzyme is important in dealing with foodstuffs because of its accelerating influence on the development of oxidative rancidity in fat and its destruction of certain vitamins. Its carotene destroying power has been the subject of a process patented by Haas and Bohn (7) to bleach bread dough.

Practically all the work on lipoxidase has been done with soybean meal. It has been found that this enzyme oxidizes higher unsaturated fatty acids such as linoleic and linolenic acid. These acids combine with at most one or two moles of oxygen, respectively, in the enzymatic oxidation. Bergström (3) found that the oxidation of linoleic acid with soybean lipoxidase followed the same general course as a metal catalyzed autoxidation of this acid. Both the metal and enzyme catalyzed reaction products exhibited maximum ultraviolet light absorption at 232 mμ and the same two monohydroxy acids, 9- and 13-hydroxystearic acid, were isolated in comparable yields from the hydrogenation products. Higher oxygenated acids were also formed in comparable amounts by enzymatic and autoxidation reactions.

The present study was undertaken to establish the disposition of lipase and lipoxidase enzymes in baked products and to determine the threshold value for the enzyme reaction products in baked bread by means of consumer acceptability studies.

Materials and Methods

The flour used in this study was a blend of experimentally milled flours having a protein content of 13.0% and an ash content of 0.44%.

The special shortenings used were COVO shortening and a hydrogenated cottonseed oil.

The straight dough procedure employed by Johnson and Miller (8) was followed. All loaves were baked in duplicate. Bread to be used for subsequent organoleptic testing was wrapped in wax paper, sealed with melted paraffin, and stored at 77° ± 1°F. until needed.

The method for determining lipase activity adopted for this study was based on the manometric determination of the carbon dioxide evolved from a bicarbonate buffer (pH 7.4) as a result of the liberation of free fatty acid from mono-n-butyrin substrate (Singer and Hofstee, 13). This procedure adopts several compromise conditions. Although the higher triglycerides of fatty acids would provide a natural substrate system, these agents are only slightly soluble in water and therefore difficult to manipulate. Mono-n-butyrin substrate is soluble in water in all proportions. The pH employed (13) is somewhat higher than the optimum of 5.2 for wheat lipase acting on higher triglycerides (Sullivan and Howe, 15) and is also well above the normal pH of 4.6–5.3 found in flour doughs. For comparative purposes, however, the results obtained under the conditions of the experiment were entirely satisfactory. The lipase enzyme preparation was prepared routinely immediately prior to analysis by extracting the source for 15 minutes at 3°C. with 10 volumes of water, followed by centrifugation at 1800 r.p.m. for 5 minutes and adjustment of the pH within the range 6.6–6.8. Ground defatted wheat germ was used as the source of active lipolytic enzymes.

Lipoxidase activity was determined by the method of Reiser and Fraps (11) as modified by Mitchell and King (9). A stock carotene solution was prepared by dissolving 50 mg. of purified carotene (11) in 250 ml. of doubly distilled acetone. The carotene substrate solution consisted of a 50 ml. portion of this stock solution plus 60 mg. of fresh Wesson oil brought to 100 ml. volume with purified acetone. These solutions were stored at −23°C. Before withdrawing aliquots, the solutions were heated on a steam cone to dissolve precipitated carotene and subsequently cooled to room temperature. The buffer solution consisted of 59.4 g. disodium phosphate dodecahydrate and 22.7 g. monopotassium phosphate (anhydrous) per liter of solution (pH 6.5). A reaction temperature of 30° ± 0.1°C. was maintained. Enzyme suspensions were made by grinding the source material in a glass mortar with a small quantity of water. After adjustment to volume the suspension was centrifuged, filtered, and analyzed for lipoxidase activity. Ground defatted soybean meal was used as a source of potent lipoxidase activity.

The saturated and unsaturated fatty acids of cottonseed oil were separated from each other by fractional crystallization. The cottonseed oil was saponified with alcoholic potassium hydroxide, acidified with hydrochloric acid, and the fatty acids extracted with Skellysolve F. Separation of the saturated fatty acids was effected at −23°C. The unsaturated fatty acids which remained in solution were freed from solvent under vacuum.

Selected shortenings were oxidized artificially by passing a steady stream of undried, filtered air through the fat which was heated to 90°C. with infra-red lamps. Samples were taken at arbitrary intervals for the determination of peroxide numbers (Wheeler, 19).

Organoleptic tests were made using six experienced judges. Tasting sessions were held at the same time and place each day and each judge was given a freshly cut slice of each bread type to which a coded number was assigned. Samples were rated independently for aroma and flavor acceptability and assigned scores ranging from 1 to 10.

Results and Discussion

The Lipase Activity of Baking Ingredients. Since lipolytic enzymes are present in several baking adjuncts, knowledge of this activity is essential to the understanding of the influence of lipase in baking and in the storage of baked products. The lipolytic activities for flour and a number of possible dough ingredients are listed in Table I. The activity is expressed in μl. of carbon dioxide released from the buffer during 30 minutes at 37°C.

TABLE I

MANOMETRIC ASSAY OF BREAD CONSTITUENTS FOR LIPASE ACTIVITY USING MONO-N-BUTYRIN AS A SUBSTRATE

Source of enzyme	Quantity of enzyme source	CO_2 evolved
	mg.	*μl.*
Patent flour	100	1.4
Malted wheat flour	100	1.4
Low grade flour	100	3.9
Malted barley (ground whole)	100	14.2
Soybean meal (fat extracted)	100	58.8
Wheat germ (fat extracted)	100	132.4
Fungal amylase concentrate	100	141.0
Yeast (compressed)	100	727.0
Yeast (dried)	100	1400.0
Pancreatin (commercial)	100	3150.0
Baked bread (containing 10% defatted wheat germ)	445	00.0

It is striking to note that compressed yeast as employed by the baker contained approximately 500 times as much lipase activity as did

ordinary flour compared on an uncorrected moisture basis. This value which was corrected for respiration was confirmed by means of the titrimetric procedure used by Sullivan and Howe (15). Thus, the 2% yeast normally added in baking would contain approximately 10 times as much fat splitting enzyme activity as the entire quantity of flour used. It would therefore appear that the addition of yeast to premix preparations would be highly undesirable. A few such preparations contain the yeast packaged separately. In consideration of the present data this technic should contribute to the shelf life of the food material.

TABLE II

EFFECT OF SATURATED AND UNSATURATED FATTY ACIDS IN STRAIGHT DOUGH BAKING PROCEDURE

Cottonseed oil	Fatty acids	Loaf characteristics	
		Loaf volume	Grain and texture
g.	*g.*	*ml.*	%
CHECKS			
0.0	0.0	805	80
3.0	0.0	855	80
3.0[1]	0.0	885	85
SATURATED FATTY ACIDS FROM COTTONSEED OIL			
2.5	0.5	840	88
2.0	1.0	830	88
1.0	2.0	800	88
0.0	3.0	785	86
UNSATURATED FATTY ACIDS FROM COTTONSEED OIL			
2.5	0.5	820	80
2.0	1.0	785	80
1.0	2.0	775	80
0.0	3.0	730	80

[1] Hydrogenated cottonseed oil.

The high lipolytic activity of wheat germ as compared to that for patent flour or even low grade flour corroborates the work of Engel (5) but is not in agreement with the findings of Sullivan and Howe (15) or Pett (10).

As might be expected, the lipase activity present in the normal dough ingredients or that provided by the addition of defatted wheat germ was entirely destroyed in the baking process. Lipase is thus nonfunctional during the storage of baked goods, and hydrolytic ran-

cidity due to enzyme action would not be expected to occur in baked goods such as rations which are normally stored for long periods of time.

The Influence of Free Fatty Acids on Baked Bread. Due to its availability, cottonseed oil rather than wheat fat was used as the source of fatty acids for this study. The same ratio of saturated to unsaturated fatty acids is present in both oils. Data in Table II obtained by the straight dough procedure show that as little as 0.5% of either saturated or unsaturated fatty acids caused marked deterioration in loaf volume. Only the unsaturated acids failed to improve the internal score, however. Similar results were obtained with the sponge procedure.

TABLE III

ASSAY OF WHEAT PRODUCTS, ENZYME PREPARATIONS, AND BREAD CONSTITUENTS FOR LIPOXIDASE ACTIVITY USING CAROTENE AS A SUBSTRATE

Source of enzyme	Quantity of enzyme source	Carotene destroyed in one hour
	mg.	%
Commercial flour	450	7.3
Patent flour fraction	450	7.6
Clear flour fraction	450	24.7
Bran fraction	450	20.6
Shorts fraction	450	43.1
Germ fraction (defatted)	100[1]	32.0
Yeast (compressed)	450	1.4
Malted wheat flour	250	1.6
Vegetable amylase concentrate	26[2]	0.0
Fungal amylase concentrate	31[2]	0.0
Cereal amylase concentrate	87[2]	2.0
Bacterial amylase concentrate	34[2]	0.0
Soybean meal (defatted)	1	67.0
Bread	900[3]	0.0

[1] 450 mg. of the germ fraction (defatted) destroyed 100% of the carotene in less than 30 minutes.
[2] Equivalent to the alpha-amylase activity in 3 g. of malted wheat flour.
[3] Contains 550 mg. flour and 20 mg. defatted soybean meal.

The inferior results obtained when the unsaturated fatty acids were used and the superior results obtained when a partially hydrogenated cottonseed oil was used may be due in part to the difference in physical state of the fatty acids. Baker and Mize (1) reported that bread doughs mixed and proofed below the melting point of coconut oil gave results that corresponded in all respects to those obtained with semi-solid or high viscosity fats. Doughs mixed and proofed above the melting point of coconut oil produced less desirable results similar to those obtained when liquid low-viscosity fats were employed.

Striking deteriorative changes were also evidenced by consumer acceptability ratings. A six member panel registered marked un-

favorable reactions and reported a lingering tallowy taste for bread baked with as little as 0.5% free fatty acid of either type. Sinclair and McCalla (12) found that the amount of free acid liberated in one sample of flour, which had been stored in a sealed can for 16 months, was equal to 1% of the flour. Bread baked from such flour would be expected to be nonacceptable.

The Lipoxidase Activity of Baking Ingredients. The presence of lipoxidase in wheat germ has been demonstrated by Sumner (17); however, no data are available concerning the amount of this enzyme in other wheat fractions or in the various possible baking adjuncts. A summary of the lipoxidase activity of a series of wheat fractions and amylolytic preparations is given in Table III. Of the materials investigated only wheat germ and soybean meal contained a large quantity of this enzyme. The small quantities of lipoxidase present in flour, however, may be sufficient to cause significant deteriorative changes if storage is extended for a long time under unfavorable conditions.

The lipoxidase activity of normal bread ingredients plus that present in an added 10% of soybean meal was completely inactivated during the baking process. Thus, this enzyme does not function during storage of baked goods and it may be concluded that any oxidation occurring during storage of baked products is nonenzymatic in nature.

The Influence of Oxidized Fats on the Quality of Baked Bread. The products obtained by air oxidation of the melted shortening were found to have a negligible effect on the physical characteristics of baked bread. COVO shortening with peroxide values ranging from 0 to 62.0 and hydrogenated cottonseed oil possessing peroxide values as high as 48.0 produced no noticeable changes in either loaf volume or grain and texture. A marked difference in flavor and aroma sensations was found, however, between the unoxidized samples and those with a slight amount of oxidation (peroxide number of 5). It was difficult to distinguish between, and especially to assign numerical scores to, bread baked with oxidized shortenings which possessed peroxide numbers varying between 5 and 62. Some of the difficulties encountered in judging consumer preference might have been eliminated had a ranking technic been used in which the rating given a certain sample depends on the other samples in the test (Bliss, Anderson, and Marland, 4). This procedure is adaptable either to untrained groups or to experienced judges.

The fact that the oxidized lipids were in the form of triglycerides may explain the difference in these results as compared to those ob-

tained by Sullivan *et al.* (16). While these workers (16) reported that the injurious effect of unsaturated fatty acids was much more pronounced when they became oxidized, it would be expected that the organoleptic reactions would be similar for oxidized unsaturated fatty acids and for oxidized triglycerides.

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SUNFLOWER SEED PROTEIN [1]

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ABSTRACT

The isolation of sunflower seed protein has been investigated to determine its probable value for industrial uses. The principal method of protein isolation was by alkali extraction and acid precipitation, and the protein isolated by this method was compared in color and yield with that obtained by salt extraction. The chlorogenic acid in the meal retarded the dispersion of the protein. Due to the presence of chlorogenic acid, the added alkali imparted a green color to the meal and to the isolated protein. Hot 70% ethanol or near absolute methanol were used to remove the chlorogenic acid, but they severely denatured the protein. The alkali-extracted protein had a nitrogen value of about 16.4%, and protein prepared from methanol-extracted meal followed by salt extraction and dialysis had a nitrogen value of 18.69%.

The continuous search for new oilseeds to help supply the oil and fat requirement of the United States has focused considerable attention on sunflowers. The extracted oil of the sunflower seed is without appreciable color and is reported to have good stability. The dehulled seeds have a pleasant sweetish taste and several nutritional investigators (2, 4, 5) show the oil-free meal to have a high nutritional value.

Milner, Hubbard, and Wiele (6) have examined the chemical composition and oil content of 28 samples, comprising four varieties of sunflower seed. Their results for the whole seed show a range of protein content (N × 6.25) for the four varieties of 18.04–21.40% and of 27.47–30.78% for the oil content.

The hulls constitute 39–46% of the seed for the varieties studied. When the hulls are removed the range of protein values is 29.4–32.3% and of the oil content 46.6–53.2%. The dehulled and oil-free meal will have a protein content (N × 5.4) of 52.0–65% as shown in Table I. We have used the nitrogen factor of 5.4 here since it is more consistent with our data for the nitrogen content of the purified protein (see Table II).

The latest publication on isolation of sunflower seed protein is that of Osborne and Campbell (7) in 1897. Earlier studies were made by Ritthausen (8) and Vines (12).

Osborne and Campbell prepared a series of 10 protein fractions from one batch of oil-free meal by salt extraction methods and found

[1] Manuscript received August 2, 1948.

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TABLE I

SOLVENT-EXTRACTED SUNFLOWER SEED MEALS

Solvent used	Moisture	Ash	Nitrogen	Protein (N×5.4)	Oil	Sugar
	%	%	%	%	%	%
Pet. ether (b.p. 30°–60°C.)	9.46	8.42	9.68	52.3	—	—
Ethanol—at room temperature	7.47	5.32	7.98	43.1	25.83	—
Ethanol[1]	10.20	7.35	10.92	59.0	1.29	—
Pet. ether followed by ab. methanol[1]	9.60	9.09	11.88	64.1	—	—
Pet. ether followed by 70% ethanol[1]	11.62	9.19	11.62	62.7	—	—
ORIGINAL SUNFLOWER SEED SAMPLE						
Whole sunflower seed	—	3.45	3.42	18.47	30.78	3.79
Hulled sunflower seed	—	4.07	5.12	27.65	48.80	6.14

[1] Temperature of alcoholic extractions 60°–70°C.

them to have essentially the same nitrogen content, that is, with a range of 18.00–18.24%. They concluded that, "the most abundant proteid of sunflower seed consists of a single globulin." Their first preparations were dark in color, owing to the presence of a substance known at that time as helianthotannic acid. In more recent studies, Gorter (3) identified this compound as chlorogenic acid. To remove the chlorogenic acid, Osborne and Campbell extracted the meal with ethanol of 0.820 specific gravity at 65°–75°C. and obtained a protein containing 18.58% nitrogen. He states that the last preparation "was freer from coloring matter than any before made," but he believed that he had not succeeded in separating it completely.

In view of possible expansion in the production of sunflowers as a farm crop it was decided to study isolation of the protein by the method of alkali extraction and acid precipitation, and to examine the isolated protein for industrial utilization. The sunflower seed used in this study was the Sunrise type.

Materials and Methods

The analyses of the solvent-extracted proteinaceous materials used for the nitrogen dispersion experiments and for protein isolation are shown in Table I, which also includes analysis of the original seed. The oil-extracting solvents were (a) petroleum ether, b.p. 30°–60°C., (b) 95% ethanol, (c) petroleum ether followed by 70% ethanol, and (d) petroleum ether followed by absolute methanol. The oil extractions were carried out in a modified Soxhlet extractor. For the hot alcohol extractions the temperature was 60°–70°C., and for the cold ethanol the temperature range was 25°–35°C.

The elapsed time for the hot alcohol extractions was approximately 16 hours and for the cold ethanol extraction, 60 hours.

The dispersion of the nitrogen compounds from the oil-free meal at various pH values was carried out by a procedure described in detail in an earlier publication by Smith and Circle (10) for studies on soybean protein. Two and a half grams of the finely ground oil-free meal and 100 ml. of the dispersing solution were placed in a 250 ml. centrifuge bottle and shaken mechanically for 30 minutes. The undispersed portion was removed in a centrifuge developing a maximum relative centrifugal force at the bottle tip of 1,975 times gravity and an aliquot of the dispersion taken for nitrogen analyses. The hydrogen-ion concentrations were determined with a glass electrode pH meter. For the precipitation experiments and protein isolation the meal was extracted in the pH range of 11.0–12.5. Sulfuric acid was used to lower the pH in the precipitation experiments; however, hydrochloric acid was used for protein isolation procedures. The dispersion and precipitation data and protein yields are based on the total nitrogen in the dehulled and solvent-extracted meal.

The nitrogen dispersion and precipitation data at various pH values for dehulled, petroleum ether extracted sunflower seed are shown in Fig. 1. In the precipitation data the meal was extracted at a meal-to-

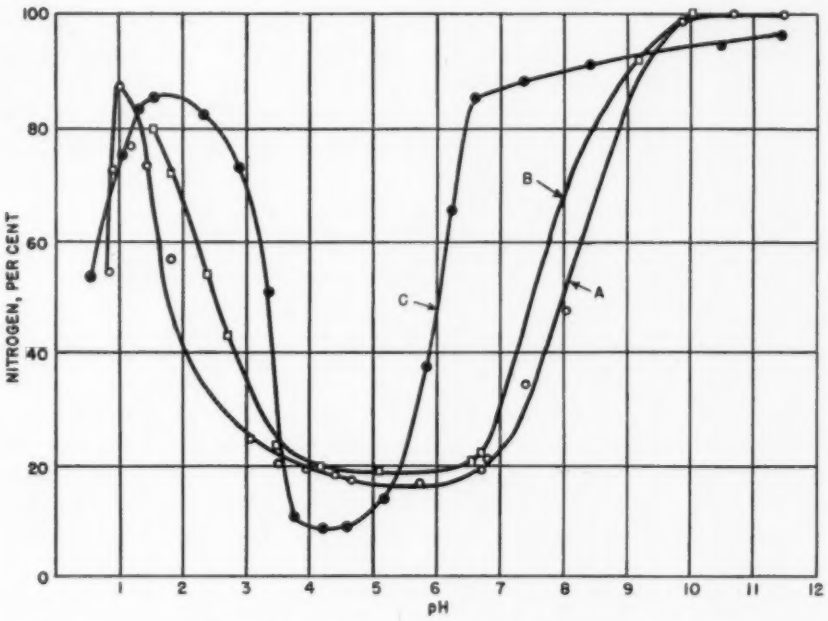

Fig. 1. Nitrogen-pH dispersion and precipitation curves for dehulled, petroleum ether extracted sunflower seed meal. Curve A, nitrogen-dispersion curve; B, nitrogen-precipitation curve; C, nitrogen-dispersion curve for soybean meal included for comparative purposes.

solvent ratio of 1:40 and at pH of about 11.0, and the undispersed residue removed in the centrifuge. The protein was precipitated from aliquots of the alkaline extract by addition of varying amounts of sulfuric acid. The precipitate was removed in the centrifuge and the difference between the percentage of nitrogen originally present in the dispersion and that left after removal of the precipitate gave a measure of the amount of protein in the precipitate. For comparative purposes, Fig. 1 also shows a pH-nitrogen dispersion curve for soybean meal taken from Smith and Circle (10).

Results

In comparing the data for the sunflower meal with soybean meal, the sunflower has a very broad minimum in the pH range of 3.0–7.0 for both the dispersion and precipitation curves. This same relationship is true also in comparing the sunflower meal curve with curves previously published for vegetable proteins such as peanut (1) and flax (11).

The water extract of the sunflower seed meal has a pH value of 6.6 but disperses only 20% of the total nitrogen. This is the same pH value as the water extracts from freshly flaked, solvent-extracted soybean, peanut, and flax meals, but the latter extracts contain 85–95% of the total nitrogen of the meals. The water extract of the sunflower seed meal contains very little protein which can be precipitated either with acid or by heating, whereas a high percentage of the protein in the other extracts is precipitated by adding acid. However, the nitrogen in the sunflower seed meal is completely dispersed at pH 10.0.

The broad minimum in the dispersion curve of the sunflower seed meal and the low water dispersibility of its protein might be attributed to the presence of chlorogenic acid, a tannin-like compound widely distributed in plant life. The dispersibility of the protein-chlorogenic acid complex at pH 10.0 is consistent with the negative reaction of vegetable tannins with proteins in the alkaline region and suggests that the complex formed between the protein and chlorogenic acid is easily dissociated.

In Fig. 2 are the nitrogen-pH dispersion curves for the sunflower seed meal which has been solvent extracted with hot 95% ethanol, for that extracted with cold ethanol, and including the protein precipitation curve of the protein from the hot ethanol extraction. The cold ethanol was such a poor oil solvent that it removed only about half of the oil.

A comparison of the curves in Fig. 2 with one another and with those in Fig. 1 shows that after extraction with 95% ethanol, either hot or cold, nitrogen solubility between pH values 3.0 and 7.0 was

lowered as much as 9.0%, and at pH 10.0 or higher the nitrogen dispersibility was lowered from 100% to 95%. The lowering of the dispersion curve between pH 3.0 and 7.0 may be explained as due to loss of soluble nitrogen compounds in the ethanol, or to partial denaturation of the protein and consequent loss in dispersibility, or both. Since there is such slight difference between the curves for the cold and hot ethanol extractions, the effect of denaturation on dispersibility is thought to be rather small. This is further substantiated by the comparatively small shift in the curve to a higher pH range

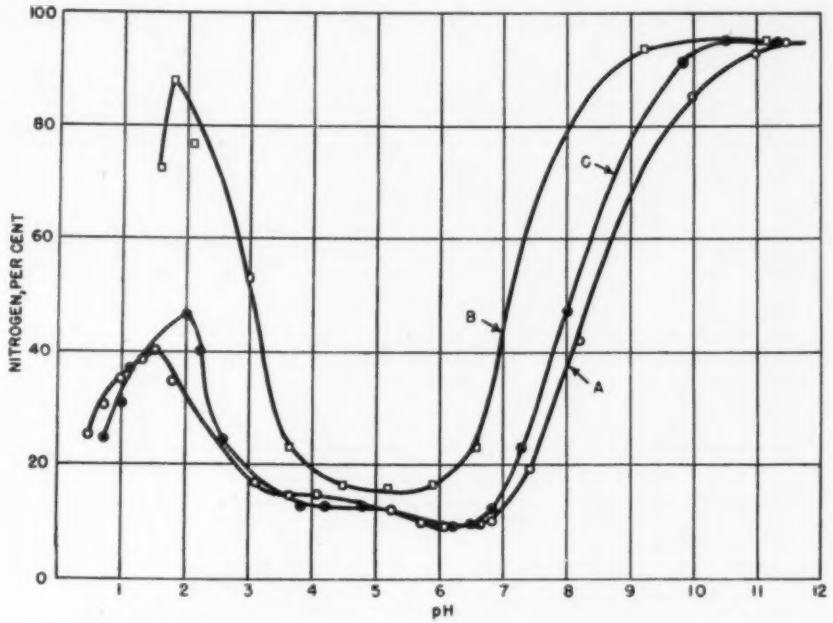

Fig. 2. Nitrogen-pH dispersion and precipitation curves for dehulled, ethanol-extracted meal. Curve A, nitrogen-dispersion curve for hot-ethanol-extracted meal; B, nitrogen-precipitation curve for protein from hot-ethanol-extracted meal; C, nitrogen-dispersion curve for cold-ethanol-extracted meal.

which would be expected to result from denaturation. The 95% ethanol did not remove an appreciable amount of the chlorogenic acid. This was shown by the color of protein isolated from the alcohol-extracted meal, which was as dark in color as that obtained from the hexane meal.

Another indication of the presence of chlorogenic acid was the bright chrome yellow color which was obtained by adding sodium hydroxide to the meal. The yellow color changed to green on air oxidation or by chemical oxidation. The rate of oxidation or color change varied with pH of the dispersion; at pH 9.0 the green color appeared in 8–10 minutes. At pH 11.5 or higher, the color changed

directly to brown. The appearance of the green or brown color can be prevented by the use of reducing agents such as dithionite salts, but the color will partly return on washing out the reducing agent and exposing the protein to the air.

In further attempts to remove the chlorogenic acid, the petroleum ether extracted meal was re-extracted with acetone, carbon tetrachloride, benzyl alcohol, and benzene but without success.

Seventy per cent ethanol and near absolute methanol removed the chlorogenic acid from the meal in about 3 hours in a Butt extractor,

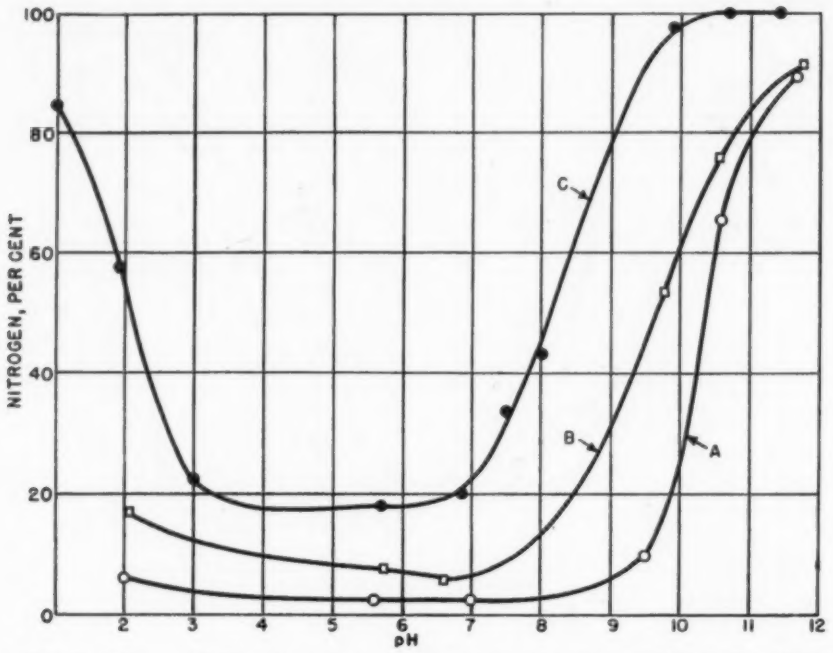

Fig. 3. Nitrogen-pH dispersion curves. Curve A, for meal extracted with hot 70% ethanol; B, for hot-near-absolute methanol; C, from petroleum ether extracted flakes included for comparative purposes.

and 70% isopropanol in about 5 hours. These extractions were made on meal which had been freed from oil with petroleum ether. Concentrations of the alcohols above and below 70% did not show any improvement in results.

The protein prepared from the above doubly extracted meals was light in color. While it was quite free of chlorogenic acid as determined by the alkali test, the protein was severely denatured as demonstrated by the pH-nitrogen dispersion curves shown in Fig. 3, where curve A is for the 70% ethanol-extracted meal, B for near absolute methanol, and C for hexane meal which was included for com-

parison. The 70% ethanol had a greater adverse effect on nitrogen dispersion than the methanol; this relationship would be expected due to the effect of the water. .

Discussion

Proteins were isolated from the sunflower seed meals which had received various solvent extraction treatments in order to estimate the extent of chlorogenic acid removal by comparing the color of the isolated proteins and to determine the relative purity of the proteins by nitrogen analysis.

In the following described methods of protein isolation, samples A, B, C, and D were from hexane-extracted meals and E was from hexane-extracted meal followed by methanol extraction.

A. Petroleum ether extracted meal—Alkali extracted twice at ratios of 15:1 and 8:1 and acid precipitated; redispersed in alkali and reprecipitated once; washed twice with water.
B. Petroleum ether meal—Alkali extracted twice at ratios of 15:1 and 8:1 and acid precipitated; redispersed in alkali and reprecipitated twice; washed twice with water after each precipitation.
C. Petroleum ether meal—Alkali extracted twice at ratios of 15:1 and 8:1 and acid precipitated; the curd washed twice with water and four times with boiling 70% ethanol.
D.° Petroleum ether meal—Extracted with 10% salt solution at ratio of 12:1 and dialyzed 7 days.
E. Petroleum ether meal followed by methanol extraction—Extracted with 10% salt solution at ratio of 12:1 and dialyzed 4 days.

The results of the protein analysis in Table II show that the alkali-extracted and acid-precipitated proteins A and B are lowest in nitrogen

TABLE II

ANALYSIS OF ISOLATED PROTEINS

Sample[1]	Moisture	Ash	Nitrogen	Nitrogen moisture ash free	Yield[2]	Color
	%	%	%	%	%	
A	9.22	0.28	14.89	16.45	32	Dark green
B	8.94	.48	14.84	16.39	31.6	Dark green
C	9.99	1.44	15.61	17.63	31.8	Light green
D	9.32	.18	15.76	17.42	22.0	Light brown
E	0	1.82	18.35	18.69	4.6	Very light brown

[1] See text for description of protein preparations.
[2] Based on weight of the oil-free meal.

content and that redispersion, reprecipitation, and water washing do not increase the purity of the protein. The color of proteins A and B is dark green. Washing the protein curd with 70% ethanol is quite effective in removing the chlorogenic acid as illustrated by sample C, although the light green color of this protein shows incomplete removal of the chlorogenic acid with this amount of alcohol washing. Protein prepared by salt extraction and dialysis of the petroleum ether meal, sample D, is quite impure in comparison to sample E, which was prepared from petroleum ether extracted meal followed by methanol extraction. Protein D was light brown. Sample E was lightest in color of all the proteins and showed no chlorogenic acid by the alkali test.

The dark color of the protein prepared from the petroleum ether and the 95% ethanol-extracted meal would exclude its general acceptance as an industrial protein. Although 70% ethanol and methanol were effective solvents for the removal of chlorogenic acid, their use would increase considerably the difficulty of protein isolation.

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MINERAL ANALYSIS OF PERUVIAN WHEAT [1]

J. A. SHELLENBERGER and W. G. SCHRENK

ABSTRACT

Wheat introduced into Peru during the period of the Spanish conquest has been grown predominantly on the same terraced mountain farms since that time. Under the circumstances it might be expected that the wheat would differ somewhat from that grown on the Great Plains Area of the United States.

Forty-six samples of wheat from various locations in Peru were evaluated for protein, ash, gluten quality, and baking quality, and the ash analyzed quantitatively for the following eight elements: potassium, phosphorus, magnesium, calcium, sodium, manganese, iron, and copper.

The total mineral content of Peruvian wheats was found to be within the limits usually reported for North American wheats; however the phosphorus, manganese, and magnesium content of the ash was lower than usually reported. Within the group of samples, only the elements potassium, iron, and copper were significantly correlated with the total ash content.

The protein content and baking quality of the samples varied widely. There was no significant correlation between total ash and protein content.

The wheat plant was not indigenous to either the North or South American continents. Wheat is reported to have been introduced first into Mexico in 1530 when one of Cortez's slaves found several wheat grains which had accidentally been mixed with some rice (5). It is probable that subsequent wheat development in Mexico and Central and South America came from this source.

Wheat appeared in Peru along with the Spanish conquest several years later. Since Pizarro found agriculture highly organized in the Inca Empire, it is probable that wheat was readily introduced into the agricultural system and has been grown on many of the same terraced areas of the Andes for the past 400 years. It is therefore of interest to compare the mineral constituents of wheat from Peru with wheats produced on newer soils and under radically different environmental conditions.

The mineral analysis of wheat ash has attracted much attention, and several excellent reviews of the literature have been given by Sullivan (9), Beeson (2), and Bailey (1).

[1] Manuscript received May 6, 1948. Presented at the annual meeting, May, 1948.
Contribution No. 149, Department of Milling Industry and No. 362, Department of Chemistry, Kansas Agricultural Experiment Station, Manhattan, Kansas.

TABLE I

DESCRIPTION AND ANALYSIS OF PERUVIAN WHEATS

Sample no.	Location where grown	Type of wheat	Test weight	Protein [1]	Ash [1]	Loaf volume
			lbs.	%	%	*cc.*
1	Celendin	White vulgare	58.5	12.1	1.70	790
2	Huanuco	White vulgare	57.9	13.7	1.69	835
3	Huanuco	White vulgare	54.6	9.5	2.56	545
4	Huanuco	Red vulgare	58.5	12.1	2.17	590
5	Huanuco	White vulgare	57.7	13.7	1.57	775
6	Huanuco	White and red vulgare	55.6	9.1	1.53	625
7	Huacho	White vulgare	59.5	11.8	1.60	700
8	Tarma	Amber durum	55.2	9.1	2.22	450
9	Concepcion	White vulgare, 5% durum	59.5	7.6	1.53	340
10	Concepcion	Red and white vulgare	57.8	9.4	1.60	350
11	Concepcion	Red and white vulgare	57.3	10.7	1.36	495
12	Cajamarca	Red vulgare	60.0	12.1	1.49	880
13	Cajamarca	White vulgare	57.9	7.6	1.70	470
15[2]	Cajamarca	White vulgare	59.8	7.8	1.68	445
16	Cajamarca	White vulgare	60.5	7.8	1.45	485
17	Cajamarca	Red vulgare	57.5	9.4	1.78	595
18	Cajamarca	White vulgare	57.5	11.3	1.62	600
19	Cajamarca	Red vulgare	61.2	9.1	1.65	440
20	Cajamarca	White vulgare	58.4	11.6	1.48	705
21	Cajamarca	Red, few white vulgare	58.2	10.4	1.29	740
22	Cajamarca	White poulard	56.2	7.9	1.31	440
23	Huancyo	White vulgare	57.6	8.5	1.48	455
24	Huancyo	White vulgare	59.4	8.0	1.35	325
25	Huancyo	Amber durum	58.2	9.1	2.14	310
26	Huancavelica	White vulgare	54.3	9.6	1.46	400
27	Cusco	White vulgare	55.9	6.3	1.77	440
28	Cusco	Red vulgare	59.2	7.9	1.41	560
29	Cusco	White and red vulgare	57.4	8.6	2.07	540
30	Cusco	White and red vulgare	59.5	10.4	1.48	535
31	Cusco	White and red vulgare	56.1	8.6	1.71	595
32	Cusco	White and red vulgare	56.4	8.9	1.54	505
33	Camana	Amber durum	57.1	10.9	2.13	505
34	Arequipa	White vulgare	58.6	11.0	1.62	585
35	Arequipa	Red and white durum	56.0	11.2	1.69	475
36	Arequipa	White vulgare	61.4	11.6	1.44	740
37	Arequipa	Hard white vulgare	60.2	12.1	1.32	750
38	Arequipa	Red vulgare	58.6	14.1	1.67	835
39	Arequipa	Amber durum	58.2	11.9	1.55	585
40	Arequipa	White vulgare	56.0	11.2	1.69	770
41	Moquequa	White vulgare	60.4	9.7	1.84	675
42	Moquequa	White vulgare	62.2	8.6	1.57	705
43	Moquequa	Amber and red durum	56.8	8.7	1.90	530
44	Moquequa	Red vulgare	58.2	11.9	1.95	735
45	Tacna	White vulgare	59.3	9.7	1.73	580
46	Tacna	Red and white vulgare	59.2	9.7	1.73	660
47	Tacna	White vulgare	48.6	9.5	1.77	605

[1] Results reported on 14% moisture basis.
[2] No. 14 was hull-less barley.

Materials and Methods

Forty-six samples of wheat were obtained from different locations in Peru (8). The location from which the samples came, the type of wheat, the protein, ash, and test weight are recorded in Table I.

The wheat samples were ground, mixed, and ashed by usual procedures. Ashing was done at 600°C. The ash was then analyzed for potassium, phosphorus, magnesium, calcium, sodium, manganese, iron, and copper. All elements except potassium and calcium were determined spectrographically. All data are reported on a dry weight basis.

Potassium was determined by a method recommended by Harris (3). A procedure suggested by Wang (11) for calcium in blood serum was modified slightly for the calcium determination. A 20 mg. sample of ash was dissolved in the buffer solution, after which the procedure of Wang was followed.

The remaining mineral constituents were determined spectrographically on a Bausch and Lomb large litrow spectrograph, using the standard solutions, spectral lines, and techniques recommended by Morris *et al.* (6) with the following modifications.

Samples were placed in solution and on electrodes as recommended by Morris (6). The image was focused on the collimating lens and electrode spacing was set at one mm. Samples were arced five minutes. The excitation source was a high voltage A.C., operating at 2,200 volts and 2.4 amperes. The sector was set at three-eighths open. All spectra were taken in duplicate and a set of standards was placed on each plate. Line densities were read with the aid of an ARL—Dietert densitometer. This is essentially the same techinque as used in the determination of the mineral content of Kansas wheat (7).

Results and Discussion

The data recorded in Table I show that the samples were obtained from representative wheat growing areas throughout Peru from Celendin in the North to Tacna near the Chilean boundary. Most of the samples were white or red vulgare types, but durum and one sample of white poulard are included. The protein, ash, and test weight are also included in Table I. The protein content varied over a wide range from 14.0 to 6.3%, and the ash content varied from 2.56 to 1.29%. There was no significant correlation between protein and total ash content. This is in contrast to the significant relationship between protein and mineral content reported by Schrenk and King (7) for Kansas grown wheats.

The average analysis of the ash of the 46 samples for the eight elements, potassium, phosphorus, magnesium, calcium, sodium, man-

TABLE II

Comparison of the Average Mineral Analyses of Different Wheats

Source	Protein	Ash	K	P	Mg	Ca	Na	Mn×10³	Fe×10²	Cu×10³
	%	%	%	%	%	%	%	%	%	%
Peru	11.9	1.94	0.40	0.14	0.042	0.059	0.0062	0.17	0.39	0.06
Kansas[1]	14.8	2.07	0.40	0.43	0.135	0.046	0.0170	0.48	0.74	0.64
Marquis[2]	15.9	2.05	0.52	0.44	0.190	0.045	0.0030	0.24	0.31	0.06
Tenmarq[3]	14.9	1.79	0.37	0.35	0.099	0.046	0.0043	0.42	0.44	0.06
Trumbull[3]	11.3	1.99	0.47	0.53	0.134	0.027	0.0085	0.57	0.65	0.10

Results reported on moisture-free basis.
[1] Data of Schrenk and King (7).
[2] Data of Sullivan and Near (10) and Howe and Sullivan (4).
[3] Data of Morris, Pascoe, and Alexander (6).

TABLE III

Comparison of the Mean Ash Content and Mineral Analysis, Including Standard Deviations of Peruvian and Kansas Wheats

Source	Total ash		Potassium		Phosphorus		Magnesium		Calcium		Sodium		Manganese		Iron		Copper	
	M[1]	S.D.[2]	M	S.D.	M	S.D.	M	S.D.	M	S.D.	M	S.D.	M	S.D.	M	S.D.	M	S.D.
	%		%		%		%		%		%		%		%		%	
Peru	1.94	.3155	.37	.0666	.136	.0377	.042	.0249	.059	.0164	.0062	.0026	.0017	.0003	.0039	.0042	.0006	.0012
Kansas	2.07	.2152	.40	.0354	.43	.0691	.135	.0154	.046	.0091	.0170	.0027	.0048	.1548	.0074	.1927	.0067	.0963

Results reported on moisture-free basis.
[1] M = mean.
[2] S.D. = standard deviation.

ganese, iron, and copper, is reported in Table II. In Table II the mineral analyses of Peruvian wheat are compared with similar data reported by Sullivan and Near (11), and Howe and Sullivan (4) for Marquis type spring wheat; the data of Morris, Pascoe, and Alexander (6) for the two red winter varieties, Tenmarq and Trumbull; and the extensive data reported by Schrenk and King (7) for hard red winter wheat. The standard deviations in the mineral analysis of the Peruvian as compared with the Kansas wheat are reported in Table III.

The total mineral content of the Peruvian wheats was found to be within the limits usually reported for North American wheats, even though great differences in variety, climate, and soil exist. The standard deviations of the total ash of the Peruvian samples compared with the analysis of wheat grown in Kansas were 0.3155 and 0.2052, respectively. Considering the wide geographical area from which the samples were obtained, the differences in type of wheat and different agricultural practices, the amount of variability in mineral matter in the Peruvian samples is not excessive.

Comparing the average values of the eight elements with similar results of other wheats, it is evident that the phosphorus, manganese, and magnesium content of the ash of Peruvian wheats is somewhat lower. In Table III the standard deviations of the elements in the ash of Peruvian wheats are compared with Kansas wheat. The magnesium, calcium, and potassium variability was greater among the Peruvian samples, but the elements iron, copper, phosphorus, and manganese showed less variability than the Kansas samples.

The degree of association between the different elements and the total ash for the Peruvian and Kansas wheats is compared in Table

TABLE IV

COMPARISON OF THE CORRELATION COEFFICIENTS BETWEEN THE TOTAL ASH CONTENT AND SEVERAL INDIVIDUAL ELEMENTS IN THE ASH OF PERUVIAN AND KANSAS WHEATS

Variables	Correlation coefficients	
	Peruvian	Kansas
$r_{A.K}$	0.37**	0.62*
$r_{A.P}$	0.11	0.68**
$r_{A.Mg}$	0.12	0.76**
$r_{A.Ca}$	−0.06	0.61*
$r_{A.Na}$	0.00	0.86***
$r_{A.Mn}$	0.14	0.46
$r_{A.Fe}$	0.68***	0.74**
$r_{A.Cu}$	0.38**	0.61*

A = Total ash.
* = Slightly significant.
** = Significant.
*** = Highly significant.

IV. Among the Peruvian samples only the elements iron, copper, and potassium were significantly correlated with the total ash content. In contrast, among the Kansas samples all the elements were significantly correlated with the total ash content except manganese. Data of Bailey and Hutchinson reported by Bailey (1) gave the following correlation coefficient between magnesium, calcium, iron, and copper content and total ash for spring wheat: $r_A.Mg = 0.75$; $r_A.Ca = 0.29$; $r_A.Fe = 0.55$, and $r_A.Cu = 0.12$. It is thus apparent that considerable variation occurs in the relationship between total ash and the content of the various elements present.

The total ash content and mineral analysis of Peruvian wheat compared favorably with similar results obtained from North American wheats. There was no evidence that three hundred years of wheat production in the same locations, under Peruvian conditions, had caused notable alterations in these common characteristics of wheat.

Acknowledgments

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THE DETERMINATION AND DEFINITION OF COLOR IN EGGS [1]

GASTON DALBY [2]

ABSTRACT

A method for the extraction of egg color by acetone, the comparison of this extract with an acidified potassium dichromate standard, and a method of reporting color in terms of a color index which is 25 times the number of milligrams of potassium dichromate equivalent to the color extracted from 1 g. of egg, has been recommended by the Committee. An acceptable commercial mixed egg will have an index of 100. The color of eggs in terms of the pigments present is discussed. Transmission curves of pure pigments compared to egg extracts and to an acidified potassium dichromate standard are shown.

The color of eggs is of commercial importance to bakers and food manufacturers who use eggs in their products. The consumer tends to judge richness of the product in accordance with the apparent amount of egg color it contains. It is not known if there is a difference in nutritional value between a light and good colored egg—the analogy to winter and summer butter may, however, suggest that there is. The value of good colored eggs to the food processor, therefore, is that of attractiveness and enhanced consumer appeal.

It is relatively common practice in food laboratories to extract the color from eggs with acetone, butyl alcohol, or other solvents, and compare the color of the extract with some standard. Turner and Conquest (3) published such a procedure. Munsey (2) in his study of pigments in egg noodles also suggested a similar type of method. One purpose of the Committee was to study the various available procedures and to recommend a standard one to the Association.

After the egg color is extracted, the next problem is to find a suitable standard with which to compare the extract. Potassium dichromate generally has been used for this purpose. Standard reproducible solutions can easily be made with this reagent, and in color it is a close match to the average pigments extracted from eggs. After the colored extract is compared with the dichromate standard the question arises as to how the color of the eggs shall be reported. Shall this be in terms of dichromate concentration, in terms of Tintometer filter factors, or percentage transmission at a specified wave length?

[1] Manuscript received June 2, 1948.
Report of the Committee on Methods for the Determination and Definition of Color in Eggs.
[2] Ward Baking Company, New York, N. Y.

The recommendation of a suitable color standard and a method of reporting egg color were also objectives of the Committee.

Materials and Methods

Apparatus: (1) Spectrophotometer, photoelectric colorimeter, Duboscq or similar visual colorimeter, Nessler tubes, or comparator tubes. (2) Filter papers: fluted. Whatman No. 12.

Reagents: (1) Acetone: technical grade or better. (2) Ammonium hydroxide: 15N. (3) Potassium dichromate soln.: 0.020% in 1.5 *M* ortho-phosphoric acid. Prepare by dissolving 0.200 g. potassium dichromate (Reagent Grade) and 102 ml. 85% phosphoric acid in water and diluting to 1 liter with water. This solution contains 0.2 mg. potassium dichromate per ml.

Weigh 5 g. of mixed eggs, or 2 g. of yolk, into a tared weighing dish. Add 5 ml. of water and mix thoroughly with a stirring rod. Transfer to a 100 ml. volumetric flask, but do not attempt at this time to remove the sample which adheres to the weighing dish. Add *immediately* about 50 ml. of acetone to the flask; stopper, and shake. Wash weighing dish with acetone and add washings to the volumetric flask. In some cases, the addition of a few drops of 15N ammonia water will be helpful in the prevention of a cloudy solution. Make up to volume with acetone and shake. After a few minutes standing, filter through Whatman No. 12 folded filter paper, and compare clear filtrate with standard solution.

Color Standard—Visual Colorimeters. Use a 0.020% solution of a reagent grade potassium dichromate in 1.5 molar phosphoric acid.

Spectrophotometers and Electric Colorimeters. Prepare standard curve with 0.010%, 0.020%, and 0.030% potassium dichromate in 1.5 molar phosphoric acid as may be required for the particular instrument available.

Nessler and Comparator Tubes. With these use more dilute standards and extract. Prepare the extract of 5 g. of mixed egg made up to 100 ml. Make a second dilution of 20 ml. of this extract to 100 ml. with acetone. Compare these extracts against standards containing 0.001, 0.002, 0.003, and 0.004% dichromate in 1.5 molar phosphoric acid.

Method of Reporting Egg Color. From comparison with the standard, the number of milligrams potassium dichromate equivalent to the color extracted from 1 g. of egg product is determined. This number of milligrams of potassium dichromate is then multiplied by the factor "25" to convert to "color index."

Collaborative Study. Two samples of frozen eggs were sent out to each committee member. The results of the collaborative study are shown in Table I.

Discussion

(a) *Dichromate Standard.* The color shade of the dichromate standard varies according to the ratio of dichromate and chromate ions present. The addition of phosphoric acid to standardize this ratio has been made in accordance with the suggestions of Kitson and Mellon (1). The absorption peak at 450 mμ makes it possible to use this inorganic material in 1.5 *M* phosphoric acid solution as a standard for the measurement of organic egg pigments in acetone since the absorption peak of the egg pigments is also at this same wave length. Transmission curves of this acidified dichromate standard, collaborative Samples A and B, Xanthophyll, and Beta Carotene are shown in Fig. 1.

TABLE I

COLLABORATIVE RESULTS OF EGG COLOR DETERMINATION [1]

Collaborator	Sample A	Sample B	Type of instrument
1	4.1	6.7	Duboscq
2	5.9	9.0	Duboscq
2	5.4	8.8	Spectrophotometer
3	5.0	8.1	Duboscq
4	4.8	7.4	Nessler tubes
5	5.0	8.0	Nessler tubes
6	5.4	8.8	Electric colorimeter
7	5.0	8.0	Comparator tubes
8	4.0	7.0	Klett
9	5.0	9.3	Duboscq
10	5.3	9.1	Duboscq
11	5.6	9.1	Duboscq
12	4.0	5.0	Nessler tubes
13	5.0	7.5	Nessler tubes
14	2.5[2]	3.0[2]	Comparator tubes
Average	5.00	8.0	
Color Index	125	200	

[1] In mg. dichromate equivalent per gram of egg.
[2] Not included in the average.

(b) *Method of Reporting Color.* For strictly scientific purposes the color of eggs in terms of milligrams potassium dichromate equivalent per gram of egg is sufficient. Since, however, in commercial practice, egg color values are used in specifications for egg purchases and the values are thus handled by nontechnical sales and purchasing departments, the Committee recommends that the factor "25" be used. Thus a mixed egg with a color equivalent of 4 mg. potassium dichromate per gram will have a color index of 100. The Committee agrees that a mixed egg with a color index of 100 is a good colored product and that this level of color is fair and reasonable to both producer and processor. A mixed egg fortified with yolk may have an index of 200. Thus a production man can readily understand that such a fortified egg

will give twice the color in the finished product as a mixed egg with an index of 100.

(c) *Egg Pigments.* There are two principal pigments present in eggs, carotene and xanthophyll, but these are so nearly identical in color that the difference between them can only be detected by means of a sensitive spectrophotometer. The nature of the transmission curves of these pure pigments, as shown in Fig. 1, demonstrates that egg color is

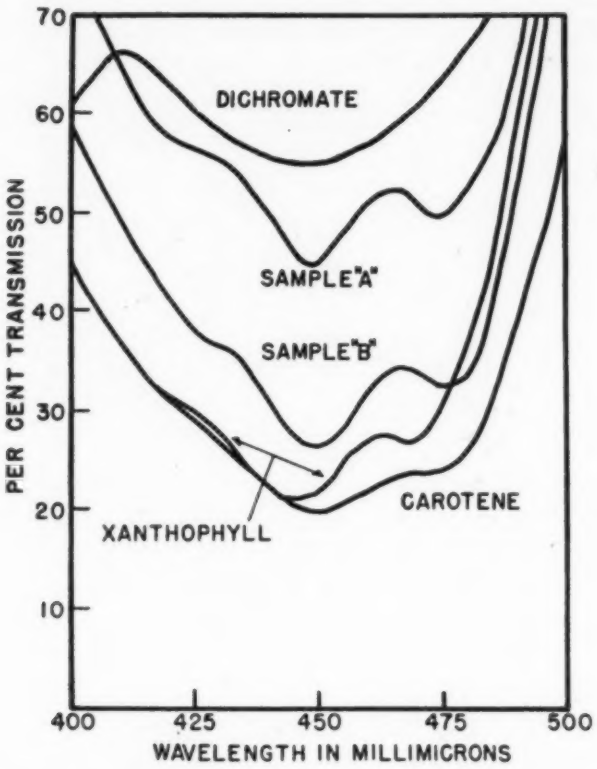

Fig. 1. Transmission curves of collaborative samples A and B, 5 g. to 100 ml. acetone; potassium dichromate, 0.02% in 1.5 molar phosphoric acid; and Xanthophyll and Beta Carotene, 4 mg. to 1 liter of acetone.

not determined by the ratio of "red" to "yellow" pigments as is so commonly assumed, but by the total concentrations of the two pigments which are almost identical in shade of color.

For many years egg color has been evaluated by many laboratories in terms of Lovibond filters. The color has been reported in terms of "yellow" and "red" units. This perhaps is the basis for the assumption that eggs contain "red" and "yellow" factors. The acetone extracts of highly colored eggs appear more red than extracts of light

colored eggs, not because of the presence of increased quantities of a red pigment, but because the higher concentrations of carotene and xanthophyll absorb more of the blue and green components of the incident light, and leave a higher proportion of red in the transmitted light. The color of neutral or acid solutions of potassium dichromate appears more red, with increasing concentrations, for the same reason.

Spectrophotometers and electric colorimeters give most satisfactory results. Visual colorimeters are next in desirability. The least desirable types of apparatus are Nessler tubes and comparator tubes.

Acknowledgments

The chairman wishes to express his gratitude to the following committee members who collaborated in this study: William B. Bradley, American Institute of Baking, Chicago, Ill.; Geo. T. Carlin, Swift and Co., Chicago, Ill.; W. H. Cathcart, The Great Atlantic and Pacific Tea Co., New York, N. Y.; D. B. Davis, Continental Baking Co., New York, N. Y.; Geo. Garnatz, The Kroger Co., Cincinnati, Ohio; R. W. Mitchell, Purity Baking Co., Chicago, Ill.; R. A. Pouchain, Tasty Baking Co., Philadelphia, Pa.; Roland Selman, C. J. Patterson Co., Kansas City, Mo.; Oscar Skovholt, Quality Bakers of America, New York, N. Y.; H. K. Steele, Fleischmann Labs., New York, N. Y.; W. H. Tonkin, Standard Brands, Inc., Terre Haute, Ind.; and N. H. Walker, Arnold Bakers, Inc., Port Chester, N. Y.

The chairman also wishes to express his appreciation to Mr. Tonkin for the preparation and distribution of the collaborative samples, to Mr. Steele for the preparation of the transmission curves, and to Mr. Earl K. Spotts of the Ward Baking Company for assistance in the preparation of this report.

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EFFECT OF ACID HYDROLYSIS ON THE RETROGRADATION OF AMYLOSE [1]

ROY L. WHISTLER and CAROLYN JOHNSON [2]

ABSTRACT

Potato amylose retrogrades from aqueous solutions more slowly than amyloses from corn and wheat starches. On acid hydrolysis, the rate of retrogradation of potato amylose increases to a maximum and then continuously decreases. Corn amylose, though lying nearer the maximum rate of retrogradation, goes through a similar increase and decrease in rate of retrogration when its molecular magnitude is decreased by hydrolysis. For both amyloses maximum retrogradation seems to occur at viscosities of $\eta sp/c$ of about 0.5.

Amylose from potato starch retrogrades in an aqueous solution at a slower rate than amylose from either corn or wheat starches. Retrogradation is the aggregation and partial crystallization of starch molecules. Solutions undergoing retrogradation may become opalescent, increase in cloudiness, increase in resistance to enzyme action, and decrease in viscosity. Amylose molecules, because of their linear nature, can coalesce and hence retrograde much more readily than the branched or bush-shaped amylopectin molecules. Even with amylose molecules, the crystallization is only partially complete, because of the steric difficulty and low probability in the attainment of perfect organization during the condensation of very long polymers. Entire molecules may sometimes crystallize in retrogradation, but it is more probable that for the most part only segments of molecules are oriented into a regular lattice to form regions of crystallinity which are capable of giving rise to a "B" x-ray pattern. Outside of these regions parts of molecules as well as entire molecules associate in more or less random fashion to produce amorphous regions. Several explanations may be given as to why potato amylose retrogrades more slowly than amylose of corn and wheat starches. One logical explanation is that the potato amylose molecules may be slightly branched, hence association is sterically hindered. Perhaps a better explanation is that the larger size of potato amylose molecules makes them so unwieldy that their

[1] Manuscript received August 16, 1948.
Paper presented before the Annual Meeting of the American Association of Cereal Chemists, Cincinnati, 1948. The subject matter of this paper has been undertaken in cooperation with the Committee of Food Research of the Quartermaster Food and Container Institute for the Armed Forces. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the War Department.
Journal Paper No. 365 of the Purdue University Agricultural Experiment Station.
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rate of coalescence and aggregation is reduced. In either case slight hydrolytic degradation of the amylose should cause retrogradation to occur more rapidly.

The present work was undertaken to determine the effect of acid hydrolysis on the rate of retrogradation of amylose.

Materials and Methods

Amyloses. Amyloses were prepared by butanol fractionation (3, 4) of good quality commercial corn, wheat, and potato starches. All amyloses were recrystallized once by dissolution in hot butanol-saturated water to form a 1% solution of amylose and allowing the solution to cool to room temperature over a period of 48 hours. Usually precipitates were collected in a supercentrifuge (40,000 R.P.M.). A portion of the separated precipitate was dried and the remainder stored under butanol at 0°–5°. To convert the precipitate to a dry powder, it was vigorously stirred into ethanol (approximately one part by volume of precipitate to five parts by volume of ethanol); the mixture filtered; and the amylose thrice more treated with ethanol. The products on drying *in vacuo* over calcium chloride were colorless, fluffy powders.

The iodine-sorbing capacity of the amyloses was measured by the method of Bates, French, and Rundle (1) as modified by Wilson, Schoch, and Hudson (6) and by Whistler and Hilbert (5). Corn and wheat amyloses sorbed 200-203 mg. of iodine per g. of amylose. Potato amylose sorbed 208 mg. iodine per g.

Hydrolysis of Amylose. Solutions of amylose were prepared by adding amylose-butanol paste to boiling water and the mixture stirred at 95°–98° for about one hour to vaporize the butanol. Solutions were adjusted to contain 1% amylose. Each was poured into individual Pyrex bottles fitted with a reflux condenser and motor stirrer and was placed in a constant temperature bath at 96°. When the solution reached the temperature of the bath, 50% sulfuric acid was added to produce a normality of 0.005. After solutions had been hydrolyzed for definite periods, the acid was neutralized with 1 *N* sodium hydroxide. An excess of butanol was added and precipitation and isolation of the amylose performed in the usual way.

Determination of Retrogradation. Air-dried amylose was weighed into a 500 ml. Erlenmeyer flask in such amount as to produce a concentration of 0.85% in 450 ml. of solution. Forty milliliters of 1 *N* sodium hydroxide were added; the flask was flooded with nitrogen and the mixture stored overnight at 0°–2° to bring about complete dispersion of the amylose. The calculated amount of distilled water was then added and the alkali neutralized with 1 *N* sulfuric acid to a

pH of 6.0–6.5. Immediately after neutralization, aliquots of the dispersion were taken for determination of initial starch concentration. Starch content of the aqueous solutions was determined by a modification of the chromic acid oxidation method of Launer (2). The main portion of the amylose dispersion was placed in a 25° constant temperature bath. After 24 hours, 40–50 ml. were removed and centrifuged in covered cups for 10 minutes at 3,900 times gravity. The supernatant was carefully decanted through a coarse sintered glass funnel and aliquots taken for analysis of amylose content by the chromic acid oxidation method as indicated above. The per cent of amylose that retrograded was calculated as the difference from the initial amylose concentration.

Determination of Viscosity. Air-dry amylose was weighed into a 100 ml. volumetric flask in such amount as to produce a solution of 0.4% when the flask was filled to the mark. The amylose was dissolved by adding 50 ml. of 1 *N* sodium hydroxide, replacing the air in the flask by nitrogen, and allowing the mixture to stand at 0°. After 0–20 minutes the samples had dissolved.

The flask was warmed to 25° and filled to the mark with 1 *N* sodium hydroxide. A portion of the solution was filtered by gravity through a medium sintered glass funnel and 10 ml. of the filtrate pipetted into a Cannon-Ostwald-Fensky viscosity tube (No. 100) and the viscosity measured at 25°. At intervals of time, thereafter, the viscosity determination was repeated with fresh samples of solution from the volumetric flask. From a plot of reduced viscosity ($\eta sp/c$) against time, a straight line was obtained which was extrapolated to zero time to give the initial viscosity of the amylose solution. To determine whether this method of extrapolation is justified, an investigation was made of the decrease in viscosity which alkaline-amylose solutions undergo with time. The results are shown in Fig. 1. A rapid decrease in reduced viscosity occurred when samples were stored in air or under nitrogen at 25°, but the decrease was slow when samples were stored at 0° under nitrogen. When the latter conditions are used, the data indicate that extrapolation to zero time is unnecessary if viscosity measurements are made soon after complete solution of the sample is attained. However, it was not always convenient to make measurements at this point, and the expedient of extrapolation to zero time was employed.

Results and Discussion

The rate of retrogradation of potato amylose is markedly affected by acid hydrolysis, as shown in Fig. 2. Unhydrolyzed amylose retrogrades from solutions of 0.85% concentration to the extent of only

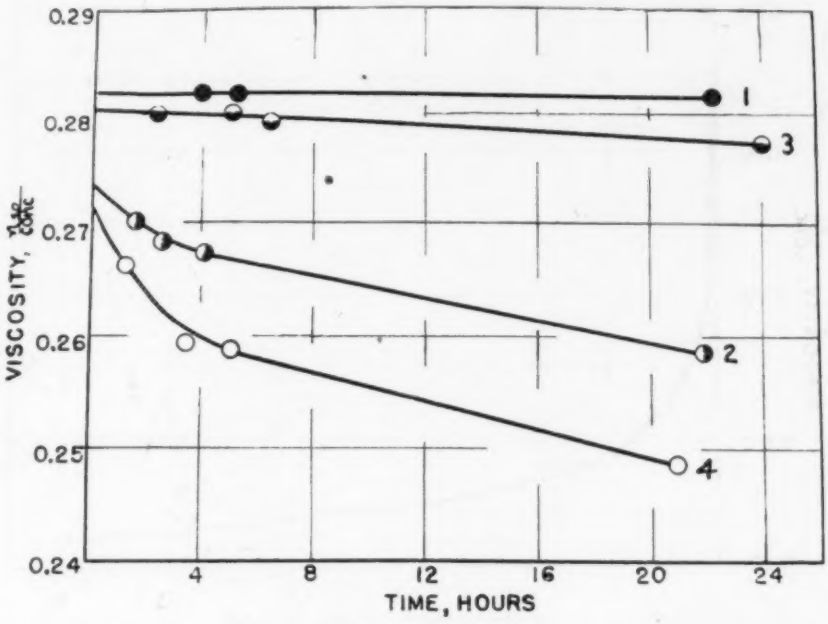

Fig. 1. Variation of viscosity with time for solutions of amylose (0.4%) in 1 *N* sodium hydroxide: (1) under nitrogen at 0°C., (2) under nitrogen at 25°C., (3) under air at 0°C., (4) under air at 25°C.

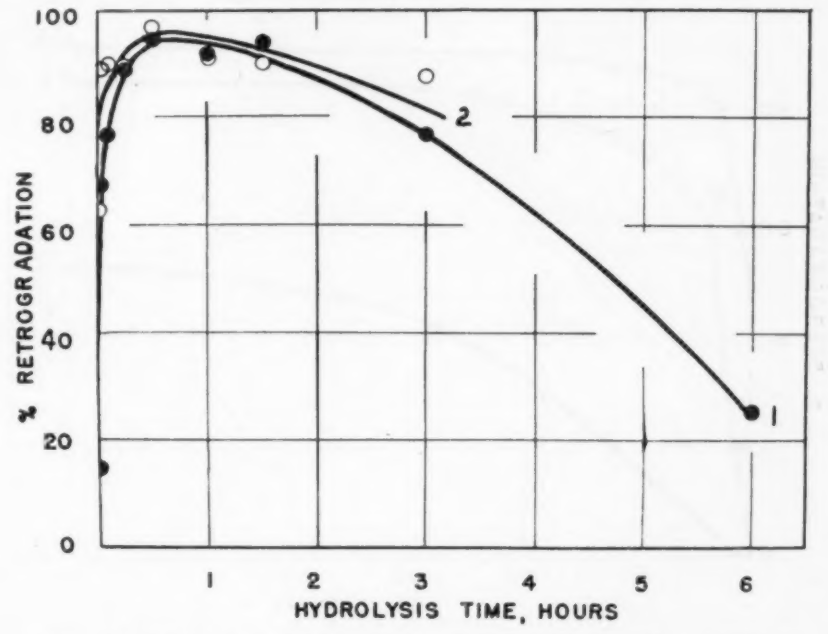

Fig. 2. Effect of acid hydrolysis on the extent of retrogradation at 25°C. (1) potato; (2) corn.

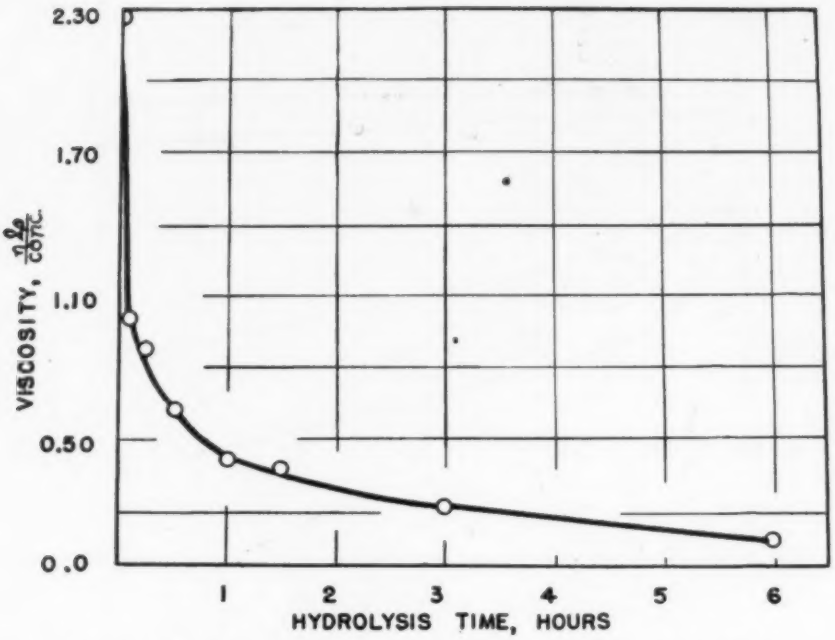

Fig. 3. Effect of time of acid hydrolysis on the viscosity of potato amylose at 25°C.

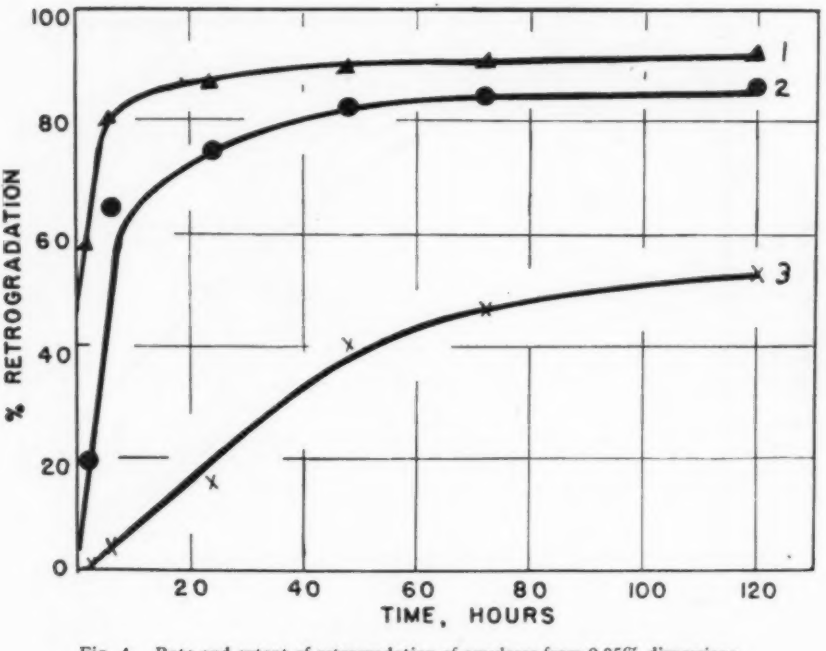

Fig. 4. Rate and extent of retrogradation of amyloses from 0.85% dispersions at 25°C. (1) wheat, (2) corn, (3) potato.

16% during the first 24 hours (see also Fig. 4). As acid hydrolysis proceeds, the rate of retrogradation increases until a maximum is reached and, thereafter, continuously decreases.

Maximum rates of retrogradation appear after 30 minutes to one hour of acid hydrolysis for the particular potato amylose used. After 6 hours of acid hydrolysis, the rate of retrogradation of the amylose again approaches the rate of retrogradation of the initial unhydrolyzed amylose.

During the hydrolysis period the molecular weight of the amylose decreases rapidly, as indicated by the viscosity data of Fig. 3. Samples which show maximum rates of retrogradation, that is, those isolated after 30 minutes to one hour of acid hydrolysis, have reduced viscosity values ($\eta sp/c$) of 0.65 to 0.35. Amylose samples which have reduced viscosities greater than these values may be too large and clumsy to undergo rapid association. On the other hand, molecules with lower viscosity values have increased solubility due to their small size.

Amyloses from corn and wheat retrograde more rapidly than potato amylose, as indicated in Fig. 4. Under comparable conditions, corn and wheat amyloses retrograde to the extent of 75.4% and 86.7%, respectively, during the first 24 hours at 25°C. They do not retrograde as rapidly as potato amylose which has been hydrolyzed 30 minutes to one hour. Their reduced viscosities of 1.06 and 0.80 suggest that they may correspond to potato amylose samples on the ascending portion of the curve in Fig. 2. For example, a comparison of the reduced viscosity of corn amylose with the data in Fig. 3 indicates that the rate of retrogradation for this amylose could be increased to a maximum value by subjecting it to hydrolysis with 0.005 *N* sulfuric acid for a period of about 30 minutes. This would be sufficient time to lower the viscosity value to about 0.5 $\eta sp/c$. When so hydrolyzed, the reduced viscosity of the isolated corn amylose is found to be 0.54. The extent of retrogradation in 24 hours is increased from 75.4% for the original unhydrolyzed amylose to 96.9% for the hydrolyzed sample. This latter value is comparable to the maximum rate of retrogradation for potato amylose (94%) exhibited in Fig. 2.

These experiments suggest that for amylose molecules there exists a critical size for which retrogradation rates are at a maximum. This maximum corresponds roughly to reduced viscosities of 0.45 to 0.56. Molecules of larger size retrograde more slowly, presumably because of steric effects resulting from their more coiled, kinked, or convoluted structure. Molecules of smaller size retrograde more slowly, presumably because of the increased solubility inherent in a lower molecular weight molecule.

This information may possibly prove of assistance to industrial workers who desire to maintain starch solutions with a minimum of retrogradation.

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COMMUNICATION TO THE EDITOR

Additional Data on Effect of Polyoxyethylene Stearate on the Crumb Softness of Bread

SIR:

In our paper, "Effect of polyoxyethylene stearate on the crumb softness of bread," *Cereal Chemistry* **24**: 346–355 (1947), the values in Table I and Fig. 1 show a substantial decrease in the rate of development of firmness of bread crumb containing this material. The data presented were obtained from tests of bread made on four different days.

Since this paper was submitted for publication, further tests have been conducted over the period February, 1947, to June, 1948, inclusive, in which bread made by the same formula and procedure as that used in the above paper, with and without the addition of 0.5% polyoxyethylene stearate, was made and tested for crumb softness at one and three days of age, as described in the paper. Comparative data for 53 pairs of observations were available and have been submitted to statistical analysis by Dr. James C. Munch, Upper Darby, Pennsylvania. Excerpts from Dr. Munch's unpublished report are submitted herewith as substantiation of the data originally presented.

"To obtain the values for the mean ($\overline{X}$) we have added together the 53 individual values and divided by 53, giving the four results shown. . . . The standard deviation, sigma $\overline{X}$, follows: then CV which represents sigma as percent of mean, for the N or 53 measurements.

"Application of the 't' test to these data indicated that there was no significant difference between the mean hardness of bread with and without 0.5% polyoxyethylene stearate after one day. On the other hand, bread made with the P.O.E.S. was significantly softer than the

TABLE I

STATISTICAL SUMMARY OF FIRMNESS OF BREAD MADE WITH AND WITHOUT 0.5% (FLOUR BASIS) POLYOXYETHYLENE STEARATE AS MEASURED BY THE BAKER COMPRESSIMETER

	Firmness as stress per 2.5 mm. strain			
	Control		0.5% polyoxyethylene stearate	
	1 day old	3 days old	1 day old	3 days old
Mean ($\overline{X}$)	96.17	185.30	83.13	143.42
Sigma $\overline{X}$	3.38	5.70	2.46	4.68
CV	3.5	3.1	3.0	3.3

control bread after three days. In the case of both breads there was a significant increase in firmness of crumb as the bread aged from one to three days and the rate of increase of the bread containing P.O.E.S. was significantly less than that of the control bread."

The average values obtained in this study compare closely with those previously reported.

Further work on the baking characteristics of this material indicates that it does have considerable dough conditioning and improving effect when used with certain types of flour not covered in the original paper. This is being investigated more thoroughly.

H. H. FAVOR and N. F. JOHNSTON
R. T. Vanderbilt Co., Inc.
East Norwalk, Connecticut

BOOK REVIEW

Cottonseed and Cottonseed Products. Their chemistry and chemical technology. Edited by Alton E. Bailey. 936 pp. Interscience Publishers, Inc., New York, N. Y. 1948. Price $17.50.

This book is a comprehensive treatise on the chemistry and technology of the cottonseed industry and will be the most important single source of reference for research and development workers in this field. Every important development in the cottonseed industry is discussed or at least referred to.

The book represents a tremendous accomplishment by the editor and 24 other eminent authorities who have contributed to the 5 sections and the 24 chapters. The complete coverage of the subject makes this a book to be referred to and studied by chapter rather than to be read completely through. A number of the chapters and substantial portions of others are of general interest and can be read easily and profitably by anyone desiring a general background of the cottonseed industry. All of the contributing authors are qualified, if not outstanding, authorities in the fields assigned to them. The editor has done an outstanding job in exercising close editorial supervision to maintain a uniformity of treatment with a minimum of duplication. The text is liberally illustrated and supplemented by charts, tables, flow sheets, photographs, selected group bibliographies, and references which exhaustively cover the subject of cottonseed and its products.

In summarizing and correlating the past and current technical achievements in the industry, several authors have also given excellent focus and direction to future needed development. Any organization or individual undertaking a research program on cottonseed or its products will do well to study the sections of this book that point out the most profitable field of additional study.

The history of the cottonseed industry makes fascinating reading. It is an industry with many unique characteristics. Although the technical advancements have been tremendous, the production of crude oil is still more an art than a science. As everyone who is engaged in it knows, he and his fellow workers comprise the finest group of people in the world. Few industries retain the rough and tough competition that still exists between operators, but few contain the fellowship and personal warm friendships that are the rule in this industry. For this reason, the development of the various trade associations amply covered in the book has an unusually important bearing on the development of the industry itself.

A listing of the sections and chapters together with the authors seems to be the only way to conclude a review of this book within a reasonable space.

A. History and Present Status of the Cottonseed Industry

I. History of Cottonseed and the United States Cottonseed Industry. By Maurice R. Cooper.
II. Production and Consumption of Cottonseed and Cottonseed Products. By Charles E. Lund.

B. Composition and Characteristics

III. Structure of the Cottonseed. By John Leahy.
IV. Cottonseed Composition—Relation to Variety, Maturity and Environment of the Plant. By W. H. Tharp.
V. Biological Processes of the Cottonseed. By Aaron M. Altschul.
VI. Pigments of Cottonseed. By Charlotte H. Boatner.
VII. Cottonseed Oil. By A. E. Bailey.
VIII. Cottonseed Protein. By Thomas D. Fontaine.
IX. Miscellaneous Constituents. By F. G. Dollear and K. S. Markley.

C. Grading and Evaluation of Cottonseed and Its Primary Products

X. Grading and Evaluation of Cottonseed. By Guy S. Meloy.
XI. Grading and Evaluation of Cottonseed Oil, Cake and Meal. By E. R. Barrow.
XII. Grading of Cotton Linters. By Guy S. Meloy.

D. Cottonseed Processing

XIII. Handling and Storing of Cottonseed. By O. H. Alderks.
XIV. Mechanical Pretreatment of the Seed. By A. C. Wamble.
XV. Cooking of Meats and Recovery of the Oil. By O. H. Alderks.
XVI. Economics of Cottonseed Crushing. By J. F. Moloney.

E. Utilization of Cottonseed Products

XVII. Processing of Cottonseed Oil. By Edward M. James.
XVIII. Edible Cottonseed Oil Products. By Howard C. Black.
XIX. Nutrition Aspects of Cottonseed Oil Utilization—The Role of Fat in Human Nutrition. By Harry J. Deuel, Jr.
XX. Nonedible Cottonseed Oil Products. By O. H. Wurster, W. J. Govan, G. J. Stockmann.
XXI. Cottonseed as a Source of Animal Feedstuffs. By Fred Hale and Carl M. Lyman.
XXII. Miscellaneous Products from Seed and Meal. By A. E. Bailey.
XXIII. Cottonseed Hulls. By John W. Dunning.
XXIV. Cottonseed Linters. By Peter Van Wyck.

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SUGGESTIONS TO AUTHORS

General. From January 1, 1948, an abstract will be printed at the beginning of each paper instead of a summary at the end, references will be numbered to provide the option of citing by number only, and date of receipt, author's connections, etc., will be shown in footnotes. Except on these points, authors will find the last volume of *Cereal Chemistry* a useful guide to acceptable arrangements and styling of papers. "On Writing Scientific Papers for Cereal Chemistry" (*Trans. Am. Assoc. Cereal Chem.* **6**: 1–22. 1948) amplifies the following notes.

Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, and all original drawings or photographs for figures. If possible, one set of photographs of figures should also be submitted. Originals can then be held to prevent damage, and the photographs can be sent to reviewers.

Titles and Footnotes. Titles should be specific, but should be kept short by deleting unnecessary words. The title footnote shows "Manuscript received . . ." and the name and address of the author's institution. Author footnotes, showing position and connections, are desirable although not obligatory.

Abstract. A concise abstract of about 200 words follows title and authors. It should state the principal results and conclusions, and should contain, largely by inference, adequate information on the scope and design of the investigation.

Literature. In general, only recent papers need be listed, and these can often be cited more advantageously throughout the text than in the introduction. Long introductory reviews should be avoided, especially when a recent review in another paper or in a monograph can be cited instead.

References are arranged and numbered in alphabetical order of authors' names and show author, title, journal, volume, first and last pages, and year. The list is given at the end of the paper. Reference numbers must invariably be cited in the text, but authors' names and year may be cited also. Abbreviations for the names of journals follow the list given in *Chemical Abstracts* **40**: I–CCIX. 1946.

Organization. The standard organization involves main sections for abstract, introduction, materials, methods, results, discussion, acknowledgments, and literature cited. Alternately, a group of related studies, each made with different materials or methods, may require a separate section for each study, with subsections for materials and methods, and for results, under each section. Center headings are used for main sections and italicized run-in headings for subsections, and headings should be restricted to these two types only.

Tables. Data should be arranged to facilitate the comparisons readers must make. Tables should be kept small by breaking up large ones if this is feasible. Only about eight columns of tabular matter can be printed across the page. Authors should omit all unessential data such as laboratory numbers, columns of data that show no significant variation, and any data not discussed in the text. A text reference can frequently be substituted for columns containing only a few data. The number of significant figures should be minimized. Box and side headings should be kept short by abbreviating freely; unorthodox abbreviations may be explained in footnotes, but unnecessary footnotes should be avoided. Leader tables without a number, main heading, or ruled lines are often useful for small groups of data.

Tables should be typed on separate pages at the end of the manuscript, and their position should be indicated to the printer by typing "(TABLE I)" in the appropriate place between lines of the text. (Figures are treated in the same way.)

Figures. If possible, all line drawings should be made by a competent draftsman. Traditional layouts should be followed: the horizontal axis should be used for

the independent variable; curves should be drawn heaviest, axes or frame intermediate, and the grid lines lightest; and experimental points should be shown. Labels are preferable to legends. Authors should avoid identification in cut-lines to be printed below the figure, especially if symbols are used that cannot readily be set in type.

All drawings should be made about two to three times eventual reduced size with India ink on white paper, tracing linen, or *blue*-lined graph paper; with any other color, the unsightly mass of small grid lines is reproduced in the cut. Lettering should be done with a guide using India ink; and letters should be $\frac{1}{16}$ to $\frac{1}{8}$th inch high after reduction.

For difficult photographs, a professional should be hired or aid obtained from a good amateur. The subject should be lighted to show details. A bright print with considerable contrast reproduces best, and all prints should be made on glossy paper.

All original figures should be submitted with one set of photographic reproductions for reviewers, and each item should be identified by lightly writing number, author, and title on the back. Cut-lines (legends) should be typed on a separate sheet at the end of the manuscript. "Preparation of Illustrations and Tables" (*Trans. Am. Assoc. Cereal Chem.* **3**: 69–104. 1945) amplifies these notes.

Text. Clarity and conciseness are the prime essentials of a good scientific style. Proper grouping of related information and thoughts within paragraphs, selection of logical sequences for paragraphs and for sentences within paragraphs, and a skillful use of headings and topic sentences are the greatest aids to clarity. Clear phrasing is simplified by writing short sentences, using direct statements and active verbs, and preferring the concrete to the abstract, the specific to the general, and the definite to the vague. Trite circumlocutions and useless modifiers are the main causes of verbosity; they should be removed by repeated editing of drafts.

Editorial Style. A.A.C.C. publications are edited in accordance with *A Manual of Style*, University of Chicago Press, and *Webster's Dictionary*. A few points which authors often treat wrongly are listed below:

Use names, not formulas, for text references to chemical compounds. Use plural verbs with quantities (6.9 g. were). Figures are used before unit abbreviations (3 ml.), and % rather than "per cent" is used following figures. All units are abbreviated and followed by periods, except units of time, which are spelled out. Repeat the degree sign (5°–10° C.). Place 0 before the decimal point for correlation coefficients ($r = 0.95$). Use * to mark statistics that exceed the 5% level and ** for those that exceed the 1% level; footnotes explaining this convention are no longer required. Type fractions on one line if possible, e.g., $A/(B + C)$. Use lower case for farinograph, mixogram, etc., unless used with a proper name, i.e., Brabender Farinograph. When in doubt about a point that occurs frequently, consult the Style Manual or the Dictionary.

CORRECTION

Magnification for Fig. 1, page 315 (September 1948) should read × 540.

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• • •

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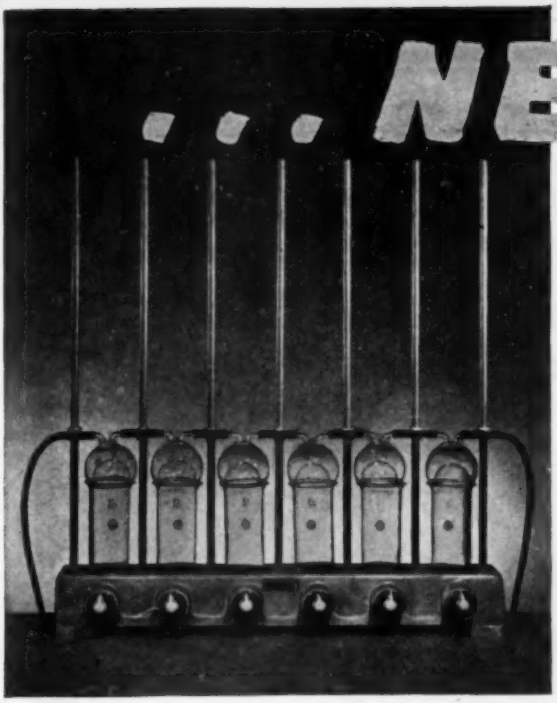

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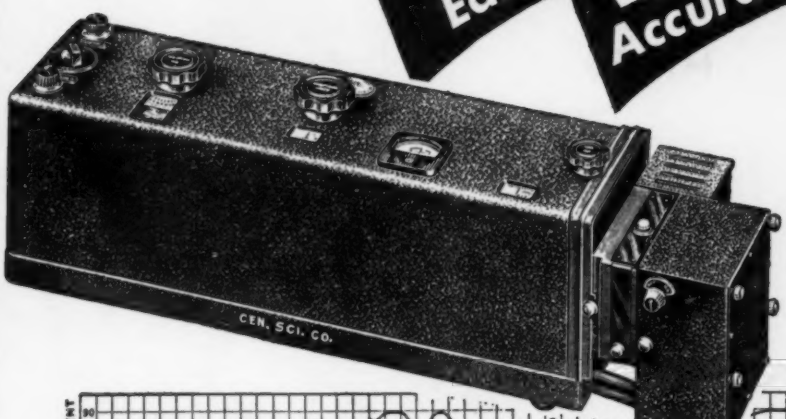

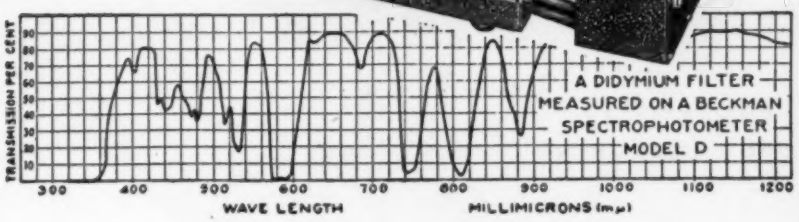

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Presentation

of the

Thomas Burr Osborne Medal

to

Betty Sullivan

by the

American Association of Cereal Chemists

May 26, 1948

Supplement to Cereal Chemistry
Vol. XXV, No. 6, November, 1948

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BETTY SULLIVAN

REPORT OF THE THOMAS BURR OSBORNE MEDAL AWARD COMMITTEE

M. J. Blish, *Chairman*

This being, for all practical purposes, the 20th anniversary of the first awarding of the Thomas Burr Osborne Medal, it seems in order to recall briefly, and especially for our newer members, the circumstances under which that award was made at the 1928 Convention in Minneapolis. The recipient of the first award was, of course, Dr. Osborne himself. Unable to attend the ceremony because of serious illness, Dr. Osborne was represented by Dr. Carl L. Alsberg who, incidentally, had served as chairman of the committee which designated Osborne as the first medalist. It was Alsberg, I believe, who obtained Osborne's consent to have the medal named in his honor, and who also was chiefly instrumental in the designing of the medal itself. A comprehensive discussion of Osborne's career and scientific achievements was contributed by Dr. C. B. Morison, after which Dr. Alsberg read a communication of appreciation which Dr. Osborne had prepared for the occasion. None of the three leading participants in that first presentation ceremony is alive today.

During the 20 years that have elapsed since the initial award was made to T. B. Osborne in 1928, there have been five recipients of the Osborne Medal, in the following order:

C. H. Bailey, 1932; M. J. Blish, 1936; C. O. Swanson, 1938; R. A. Gortner, 1942; J. C. Baker, 1945.

Today's ceremony marks the seventh awarding of the Thomas Burr Osborne Medal for distinguished contributions in cereal chemistry. In recognition, and in grateful appreciation, of her distinguished scientific contributions in the field of cereal chemistry Dr. Betty Sullivan has been chosen as our seventh Osborne medalist.

Aside from her accomplishments in fundamental research, Betty Sullivan has contributed immeasurably to the advancement of cereal technology, and to the welfare of our association, by the many and varied services she has rendered, including those of executive, administrative, technical, advisory, and editorial character. Although these services

have no bearing upon eligibility for the Osborne Medal award, our appreciation of them, and of the cheerful and friendly manner in which they have been performed, cannot help but add substantially to our pleasure and satisfaction in witnessing today's presentation ceremony.

We will now listen to an account of Betty's career and scientific contributions, as presented by George Garnatz.

BETTY SULLIVAN—FRIEND AND SCIENTIST

GEORGE GARNATZ

When I was invited to review the life and recount the accomplishments of the medalist upon this occasion, I readily consented because of the warm friendly esteem in which I hold Betty Sullivan and because of the privilege implied by the invitation. After doing so, however, I experienced an immediate reaction to the effect that though I have known Betty for a number of years (which I will indicate discreetly as being adequate to form a positive and altogether favorable knowledge of her personality, character, and ability), I was struck by how very little I knew about her prior to the crossing of our paths. It is not surprising, therefore, that I turned in other quarters for material, and at this point I acknowledge the collaboration of Miss Marjorie Howe and Dr. C. H. Bailey in the preparation of this material.

Today marks the first time the Osborne Medal has been awarded a woman and I want to express the hope that, in the course of time, other women will be thus recognized. Then, to those who succeed me in this assignment will also fall the intriguingly pleasant task of inquiring into the life of a member of the opposite sex. Let me hastily assure Betty that some vestige of chivalry still is a part of my make-up and consequently I do not intend to reveal everything I have learned. By the same token I must be careful to make sure that Betty's age remains inviolate and, so, I have deliberately garbled the chronology in this presentation, to make it difficult for anyone to ascertain any more than that Betty is enfranchised to vote. But I must get along with my story.

I suppose it is not unusual for parents to contemplate their first-born and speculate over what life has in store for them. What hopes, aspirations, and wishful thinking enter into such speculations can be grasped readily only by those of us who have already gone through this experience. There is no reason to believe that it was otherwise with Thomas J. and Blanche Guilbert Sullivan when there graced their home shortly after the turn of the century, on a certain May 31, their first-born, a baby girl, Betty. Whatever was projected in their minds' eyes, there is reason to believe that they were not aware that she was destined to

take her place beside the distinguished men who have furthered research in cereal chemistry by their important contributions.

The hopes we cherish for our children frequently become subjects of great anxiety as the young life unfolds and develops patterns of behavior that appear to be the antithesis of what we have in mind. In this respect Betty was no exception. She was different from the other little girls in her neighborhood in that, with her younger brother, she participated in all the activities of the boys. She was often referred to as a "tomboy." It was not an uncommon sight to see her wearing a tassel cap to which was attached a racoon tail or to see her in a vacant lot kicking a football. As a matter of fact, her reputation as a baseball pitcher followed her through her college days. Since she came of virtually pure Irish stock, it is not surprising to note that her inheritances included pugnaciousness and wit, with the latter inclined toward practical jokes. Thus, it was she gained a reputation, deserved or otherwise, as a superior fighter and she could spit farther than any challenger. The strategic application of stink bombs and the preparation of unorthodox pink lemonade for faculty teas have been credited to her, but culpability was never fully established because, inconsistently enough, she utilized her feminine wiles to win exoneration. Fortunately for her reputation and the peace of mind of her associates, these traits, with maturity, were converted into the aggressive initiative, the warm friendly humor, the dispassionate viewpoint, and the sportsmanlike manner she has applied to her work and her dealings with people, to the end that the former has won for her high recognition and the latter a host of friends.

Betty Sullivan is truly a product of Minneapolis. It is not only the city of her birth, but it has been there also that she has received practically all of her formal education and has practiced her profession. She received her elementary training in the Minneapolis public schools and then graduated from Central High School at the age of sixteen. She entered the School of Chemistry of the University of Minnesota. There she came to the attention of Dr. George Frankforter who, at that time, was Chief of the Department of Chemistry. He recognized the potentialities residing in her and took it upon himself to counsel and direct her. Thus was formed a friendship that undoubtedly was an influence in Betty's life and which endured throughout the years. Before Dr. Frankforter died in 1947 he was privileged to note the accomplishments of another one of his students for whom he had provided a significant measure of inspiration. In due course Betty met the requirements for the degree of Bachelor of Science and thus completed her undergraduate

work. Under Dr. Frankforter's direction, as her senior thesis, she worked on the "Polymerization of Pinene." Here was obtained the first indication of her ability at research, for her thesis was considered of such merit that it was presented by Dr. Frankforter at the 1922 meeting of the American Chemical Society.

The circumstances under which Betty became associated with the Russell-Miller Milling Company are interesting to note and serve to remind us how the recommendations of our friends frequently affect our careers profoundly. In 1922 Professor Harry Snyder was chief chemist for Russell-Miller and had occasion to call in Dr. Frankforter for consultation. The latter turned to Betty for assistance with some of the analytical details, the upshot of which was that within a few weeks Professor Snyder hired her as an assistant chemist. Thus was begun unauspiciously an association which has flourished with the advancing years, and there came into Betty's life another man whose research-mindedness undoubtedly left its impressions upon her. Of these days, Betty says, herself, that Professor Snyder exhibited considerable courage in retaining her, since previous to that time, no woman had been employed in the mill's operating plants. She says, too, that it was the only time she ever found being a woman was a handicap rather than an asset.

In 1924 Betty went to France on an International Education Scholarship and studied at the University of Paris. There she took courses under such outstanding lecturers as Gabriel Bertrand, Madame Curie, Maurice Javillier, and M. Schoen. She transferred to Pasteur Institute the next year and worked for Auguste Fernbach, chief of the fermentation division. I was able to wring from Betty the confession that she did not neglect the night life of Paris which, at that time she vaguely intimated, was wonderfully exciting. When pressed for further details she became tight-lipped and reticent. All I could make out was some reference to strumming a mandolin at the Parisian versions of what we designate as night clubs and, which she was careful to assure me, were not as I thought they were at all.

Upon returning home in 1925, with only a few cents in her pocket, but with her mind stimulated by her scientific studies abroad, she resumed her association with Russell-Miller. In 1927 Professor Snyder died and, as this scientific partner left the scene, there entered into the picture another man who through his foresight, constructive help, and encouragement was responsible for providing Betty with her opportunity, by appointing her chief chemist. This man was Harry Helm, at that time president of Russell-Miller. When he died in May, 1947, a loyal

friend and supporter had been lost to Betty, but Mr. Helm was privileged to see his confidence in Betty rewarded by noting with what prestige she was regarded professionally, while at the same time advancing to the position of vice-president and director of her company.

With the assumption of the duties of chief chemist Betty Sullivan was launched on a career wherein she has been successful to a high degree and in the pursuit of which she has manifested a capacity for work, has demonstrated much versatility, and has embarked upon a succession of research activities, interwoven with technological tasks, which have earned for her the recognition being accorded her today.

As her experience expanded, Betty felt the need for additional post-graduate study and so decided to go back to school while continuing at work. She majored in biochemistry at the University of Minnesota under the guidance of Dr. C. H. Bailey as advisor and minored in organic chemistry. Having successfully met the requirements, she received the degree of Doctor of Philosophy in 1935.

Ever since, she has been associated with the Russell-Miller Milling Company where the work of herself and her staff has been effectively helpful in producing products uniformly good functionally, in extending technical services to users of the products, in the development of better or new products, and in bringing prestige to themselves and their company by the high quality of the researches they have published. In her capacity as director of these activities Dr. Sullivan has given full play to her ability, her sense of humor, and her kindly, genuine interest in others. Her tireless capacity for work is a challenge to those who work with her. When things go wrong or when the work becomes burdensome, some humorous remark of hers lightens the task. Her kindly interest generates a reversible reaction wherein she gives of herself to others and receives from them their wholehearted cooperation, their liking, and their loyalty. Betty, herself, is fully appreciative of the assistance she has received from others and is generous in giving credit wherever credit is due. In fact, she is so inately modest, she tends to discount her own contributions in relation to those of others.

There was an era in which chemists devoted themselves so single-mindedly to their profession, they embalmed themselves in an atmosphere of isolation. They did not concern themselves with other matters and thus gained for themselves the reputation, among ordinary folks, of being "queer" and introverted. Fortunately that era is passing and chemists, along with scientists and engineers generally, are becoming increasingly socially conscious. Dr. Sullivan is an example of this new type of

scientist, for her interests are broad. True, her main interest is research and her motivations do not stem from a desire for material things. Still, she has an interest in music (she admits to playing the violin, though only moderately well), she indulges in scholastic philosophy, and reads extensively. As one contemplates another facet of her personality, it is observed that she is also the outdoor type (an inheritance from her "tomboy" days, no doubt), indulging in swimming and tennis. She thoroughly enjoys living and says that humorous instances have occurred so frequently, it is difficult to single out a few for recounting here.

Betty's activities in affairs related to her profession are numerous. Only the high lights can be reviewed. She is a member of Sigma Xi, the American Association for the Advancement of Science, the American Chemical Society, the American Society of Bakery Engineers, and La Societe de Chimie Biologique. She has served as vice-president and treasurer of the Minnesota Section of the American Chemical Society. Since 1942 she has been chairman of the Technical Advisory Committee of the Millers' National Federation. In this capacity she has served ably and tactfully. Her influence was persuasive and induced the millers and cereal chemists to provide enabling financial support for the publication of the Cereals and Cereal Products Section of Biological Abstracts. She also gave a good account of herself as an expert witness in court, although in the case I have in mind, the judge bore the name of Sullivan too. Dr. Sullivan is listed in American Men of Science and is serving currently as treasurer of the Associates of the Food and Container Institute for the Armed Forces.

She has been a member of the American Association of Cereal Chemists for some time and has contributed importantly to its activities. She has served on numerous committees and in many instances functioned as chairman. She has progressed through the chairs of the Association, so to speak, and in 1943–44 served as President. At present she continues actively in the work as a member of the Editorial Board of Cereal Chemistry and the Monograph and the Abstract Service Committee. Within the recent past she has been identified with the History, Nominating, and Revision of Cereal Laboratory Methods Committees.

But these things, while contributory, are not the reasons why we are honoring Dr. Sullivan today. We are bestowing upon her the highest recognition within the province of our Association for her outstanding research contributions to Cereal Chemistry. These contributions started with the establishment of the scientific partnership between Professor

Harry Snyder and herself, out of which stemmed a series of publications based upon their studies of the moisture in wheat and flour. Their findings exerted a profound effect not only upon the evolution and understanding of the methods themselves for determining moisture, but also upon the standards for flour that were reviewed and rewritten during that period.

Upon her return from abroad, Betty Sullivan engaged in a series of studies involving the inorganic constituents of wheat and flour, their quantitative determination, and relation to flour properties. This led to a consideration of certain groups of complex organic constituents, including the compound lipids, and thence to the entire group of lipids present in wheat products.

At this point she matriculated in the Graduate School of the University of Minnesota and this interest in the lipids was expanded to afford a basis for the doctor's dissertation. Her thesis entitled "The Lipids of the Wheat Embryo" was made the basis of two articles that were published in the Journal of the American Chemical Society in 1936.

This interest in the lipids and related substances has continued in the intervening years and has resulted in a series of papers which constitute the standard sources of information concerning them and identify Dr. Sullivan as the principal authority on that subject.

Glutathione, oxidizing and reducing agents in flour, the application of the dropping electrode to the study of oxidation-reduction systems in flour, and related issues were made the subjects of special study by Betty Sullivan in recent years, which have been recorded in a series of publications.

The technological significance of these and other constituents and systems was not overlooked. The extent of and manner in which Dr. Sullivan applied the latest and most refined techniques in all of these intricate studies and analyses were notable and most commendable. In consequence, the findings were accurate and complete to a singular degree. They exemplify the determination of this investigator that no findings will be accepted and recorded which are not derived from the most accurate and appropriate practices known to modern science.

Besides these accomplishments, Betty Sullivan has had patents granted her on two devices she has conceived, namely, a "Flour Improver" and "Accelerated Moisture Conditioning and Milling of Grain." She was also invited to write the chapters on "Esterases in Relation to Milling and Baking" and "Oxidizing Enzyme Systems of Wheat and

Flours" for the Cereal Chemistry Monograph, *Enzymes and Their Role in Wheat Technology*.

In 1944 in Minneapolis, I met Blanche Guilbert Sullivan when, with a mother's natural pride, she came to see Betty preside as president of the American Association of Cereal Chemists. The infirmities that come with age prevent her from being present today. I am sure, though, that she is with us in spirit and in her heart recognizes that her aspirations for her first-born have been realized fully, even though the accomplishments of her daughter may not be in the fields she anticipated. I understand that Mrs. Sullivan was an accomplished musician, and, as a master of one of the arts, was constantly puzzled over the utility of her daughter's chosen field of endeavor until Betty demonstrated her talents quite tangibly one day by repairing the mechanical refrigerator in their home. Now, with the confirmation afforded by this event, I am sure that Betty's mother has realized what all conscientious parents aspire to, that is, to witness in their children the attainment of such character and accomplishments as win for them the respect and recognition of their fellowmen.

Thus, is brought up to date the review of the medalist's life and accomplishments. Were I as familiar as I should be with the great passages in literature and poetry, this would be the logical place at which to insert a concluding quotation. But, since I am what I am and there is only one Betty Sullivan, I must revert to the vernacular and say, "What is going to happen to Betty Sullivan couldn't happen to a nicer (or a more worthy) person."

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PRESENTATION OF THE MEDAL

R. M. SANDSTEDT

I feel highly honored to be privileged to represent the American Association of Cereal Chemists on this occasion of the presentation of the Osborne Medal. As Mr. Garnatz has just said, "The Osborne Medal is the highest award which the Association bestows."

Dr. Betty Sullivan, in token of our respect for your scientific achievements and in appreciation for your contributions to our profession and to our Association, we are pleased to present you with the Thomas Burr Osborne Medal.

ACCEPTANCE OF THE OSBORNE MEDAL BY BETTY SULLIVAN

Mr. President, Doctor Blish, Mr. Garnatz, Fellow Chemists, and Guests:

It is with both humility and appreciation that I accept the Thomas Burr Osborne Medal. The humility is occasioned by the fact that the only sound knowledge I possess is the realization of my own little worth. The appreciation is felt because I now have the opportunity to acknowledge my debt to so many who have helped me so generously: to my mother and father, to my teachers—Monsignor Casey, Professors Frankforter, Snyder, Hunter, Javillier, and our own Dean Bailey—to all the officials of my company, particularly the late Harry Helm, to my colleagues in the American Association of Cereal Chemists, especially those who have worked with me in the Russell-Miller laboratories—Marjorie Howe, Frederick Schmalz, Grant Astleford, William Richards, Edgar Joseph, Roy Gannon, Guy Foley, Martin Sticka, Ellwood Fisher, George Trum, Bernard Lea, and Dean Weber. Gratitude is the memory of the heart and, in a real sense, this medal belongs to all these people. In their names, I thank you.

THE MECHANISM OF THE OXIDATION AND REDUCTION OF FLOUR

BETTY SULLIVAN

The mechanism of the action of oxidizing and reducing agents on flour is a challenging subject for speculation and experiment. Shakespeare, in one of his plays, has said, "And many strokes, though with a little axe, hew down and fell the hardest timbered oak." Some strokes will be given here, many have been given before, but, at the conclusion, the oak still will be standing, although, I hope, somewhat nearer being felled.

There is a definite optimum oxidation potential for the best baking characteristics of every flour and it is seldom indeed that this potential occurs naturally without long aging of either the flour or the wheat from which it is milled. It is well recognized that certain oxidizing agents, such as bromates, iodates, persulfates, nitrogen trichloride, chlorites, chlorine, and chlorine dioxide, improve the baking quality of many flours, when employed at the proper level, whereas certain reducing substances, such as sulfhydryl compounds, sulfites, and cyanides, cause doughs to become sticky, lose their gas-retaining ability, and produce poor baking results. Untreated flours usually are "green," or underoxidized. This condition is manifested in the dough by inferior physical properties, such as softness and extreme extensibility, and, in the baked product, by poor volume, round, thick cell walls, and little break or shred. The longer the extraction of the flour, the more extreme are these effects. Reducing agents intensify this greenness. Treatment, with certain oxidizing agents at their optimum level, improves the elasticity and handling characteristics of the dough. Bread or other baked products have maximum volume, thin, elongated cell walls, and greater sheen and palatability. Overtreatment with oxidizing agents gives inferior results, shown mainly by extreme shortness and buckiness of the dough, poor grain and texture, and a wild break.

Adequate proof of the theories advanced to explain these reactions has not yet been provided, although there are some investigators who consider that experimental evidence is sufficient to demonstrate that the

changes, observed on the addition of the particular oxidizing and reducing agents, can be explained on the basis of the activation or inhibition of the proteolytic enzymes of flour. This explanation provides only a partial answer to the whole problem.

In this address, my ideas on this problem will be presented, based mainly on work, published and unpublished, done in the Russell-Miller laboratories, but necessarily relying also on the voluminous literature from other sources.

Starch, Lipids, and Proteins

Let us consider first the three main substances in flour—starch, lipids, and proteins.

Starch. Although the starch molecule can be oxidized by a variety of reagents, it is conceded that starch is not involved significantly in the problem under discussion. Starch removed from flour by washing, treated separately with improving agents or by long aging, and then reconstituted with gluten and baked, shows no effect. Furthermore, alpha- and beta-amylases are not implicated.

Lipids. The lipids undoubtedly play a minor, though significant, role. Our results on a wide variety of flours show that the lipids have a pronounced effect on the elasticity of gluten and dough. The removal of the major portion of the lipids from the flour, by ethyl or petroleum ether, results in a tougher gluten and a buckier dough. Moreover, the partial extraction of the fat constituents from some low-grade flours enables oxidizing agents to act with greater effect than is the case with unextracted flour, as Bungenberg de Jong (5) has shown. This probably is a purely physical effect. However, it has been demonstrated (22) that iodate, bromate, and, to a lesser extent, nitrogen trichloride cause definite chemical changes in the lipids of the gluten (where most of the fat is held) from a fermenting dough. Glutens were washed from untreated, bromated, and iodated doughs containing 0.25% yeast, after 18 hours of fermentation. It was found that not only was there a loss in the total fat-soluble materials from the treated flours, but a large loss of nitrogen and sulfur in the lipids of these glutens. Iodate produced the greater loss, with the disappearance of 99% of the nitrogen and 36% of the sulfur. The sulfur-containing crystalline lipoprotein, isolated by Balls and Hale (1, 2) from a petroleum ether extract of flour, may be involved in these changes. In spite of this and other evidence that demonstrates that the lipids should not be ignored in a consideration of the mechanism of oxidation and

reduction in flour, it can be proved that neither the glycerides, phosphatides, nor other fat-soluble compounds associated with them is primarily responsible for the effects observed on oxidation or reduction of flour. Ethyl or petroleum ether-extracted flour shows nearly full response to the usual improving agents. Although neither solvent completely removes all of the phosphatides, a fair portion is extracted in the presence of the glycerides. So, we can eliminate the lipids as the main causative group.

Proteins. It is the proteins of flour and the peptide chains associated with them which are responsible for the major effects observed on oxidation and reduction. Ample proof for this statement can be found in the literature. Now it is evident that, in order to produce the effects observed in fermentation and even in some chemically leavened products, there must be some groups in the protein capable of being oxidized and reduced with the reagents and conditions commonly employed in baking. There are several such groups, the more likely ones being the sulfhydryl, disulfide, indole, and thio-ether groups.

With the amounts and conditions under which certain oxidizing agents act as flour improvers and certain reducing agents intensify the underoxidized state, it long has been our opinion that the sulfur-containing groups in the peptide structure are the most logical points of attack.

The Sulfhydryl Group

Let us consider first the sulfhydryl group. In 1936 in our laboratory, the tripeptide, glutathione, was isolated from wheat germ (20, 21). It was found to be present also, in much lesser amounts, in bran and, of course, in shorts by virtue of the presence of both germ and bran in this fraction. Probably because flour is not completely free from bran or germ specks, some investigators have assumed that glutathione is present in minute amounts in flour and that oxidizing agents act on the sulfhydryl group of this compound or that glutathione is present in flour and exerts its effect as an activator of a proteolytic enzyme. These assumptions are unwarranted until glutathione is identified in flour. Except for recently milled, lower-grade flours, water extracts of flour do not give a clearly positive nitroprusside test; neither do extracts made with a number of other solvents and dispersing agents. Polarigraphic studies of various extracts of underoxidized flour have failed to reveal the anodic wave characteristic of reducing groups (14). A pronounced wave is given only by a sodium hydroxide extract of flour or the gluten

washed from it, because sodium hydroxide splits the disulfide linkage of cystine.

Nevertheless, it can be shown by a variety of titration techniques that reducing substances are present in minute amounts in flour and their quantity increases with the decreasing grade of flour, from patent to the low grade.

The fact that a negative test for the sulfhydryl group is obtained with nitroprusside on the water extract of many flours which respond to oxidation is no proof that sulfhydryl groups are not present. There are both free and masked sulfhydryl groups in many proteins. The various oxidizing and alkylating reagents, commonly employed for the identification and measurement of the sulfhydryl groups, act in a different manner depending on the position and availability of these reducing groups. In the detection of sulfhydryl enzymes, Barron and Singer (3) believe that there may be native protein molecules in which, due to the particular stereochemical structure, the individual sulfhydryl groups are not sufficiently close together to form an S–S bond. In such circumstances, the oxidizing or alkylating agent might be ineffective in detecting sulfhydryl groups. With the techniques thus far employed in our laboratory for the measurement of reducing substances, such as electrometric titration, addition of excess iodine, bromate, iodate, o-iodosobenzoic acid or its sodium salt, and back titration with thiosulfate, there has been no assurance that any of these reagents provides quantitative data for all the sulfhydryl or other reducing groups present in flour. We do not know if all the reducing action is due to sulfhydryl-containing compounds or, if it is, in what compound the sulfhydryl group is present. It is worthy of mention that o-iodosobenzoic acid, iodoacetic acid and its derivative, iodoacetamide, all reagents acting on the sulfhydryl group, are good flour improvers, as Table I indicates. Some of these reagents are more specific than ferricyanide or iodine, for the detection of the sulfhydryl group. Yet the sodium salt of p-chloromercuribenzoic acid, stated by some investigators to combine with all the sulfhydryl groups in native protein, shows no improving action. This may be due to its relative insolubility.

The beneficial effect of these oxidizing and alkylating reagents strongly indicates that sulfhydryl groups are present in flour. Meyers and Working (15) reported that they could obtain no indication of either reactive or unreactive sulfhydryl groups in the gluten proteins, and no free sulfhydryl groups were found in the soluble proteins. They did find definite indications of the presence of unreactive sulfhydryl

groups which were liberated on denaturation of the soluble protein. We have found that a positive nitroprusside test is obtained on the water extract of flour only after reduction with sodium cyanide, which undoubtedly is due to reduction of cystine. The cystine content of the particular flour used was 0.29% and, of this amount, 24% was found in the water extract. Double the amount of reducing substances was liberated on heating the water extract. We also have found that, on heating gluten with 2-6 dichlorphenol-indophenol, there is perceptible reduction at the surface of the gluten (22).

In spite of the fact that no free sulfhydryl groups have been demonstrated in all flour that requires oxidation, there is strong presumptive evidence that masked sulfhydryl groups are present, since flour does respond to a wide variety of reagents capable of oxidation, alkylation, or mercaptide formation of the sulfhydryl groups. It remains to

TABLE I

EFFECT OF CERTAIN OXIDIZING AND ALKYLATING REAGENTS ON BAKING CHARACTERISTICS OF FLOUR [1]

Sample	Treatment	Dough quality	Volume	Crumb color	Grain and texture
	IODOACETIC ACID				
Stfd. stght.	None	Elastic	100	97	100
Stfd. stght.	0.0005%	Elastic	104	97	101
Stfd. stght.	0.0010%	Slightly tough	108	97	100
Stfd. stght.	0.0020%	Tough	112	96	97
Stfd. stght.	0.0030%	Very tough	107	96	97
	IODOACETAMIDE				
Stfd. stght.	None	Elastic	100	97	100
Stfd. stght.	0.0005%	Elastic	108	97	100
Stfd. stght.	0.0010%	Slightly tough	116	97	98
Stfd. stght.	0.0020%	Very tough	110	96	97
Stfd. stght.	0.0030%	Very tough	106	96	97
	O-IODOSOBENZOIC ACID				
Stfd. stght.	None	Elastic	100	97	100
Stfd. stght.	0.0005%	Elastic	102	97	100
Stfd. stght.	0.0010%	Elastic	105	97	100
Stfd. stght.	0.0020%	Slightly tough	107	97	100
Stfd. stght.	0.0030%	Tough	110	97	99

[1] All treatments were added directly to the flour. Flours were baked by the straight dough method using following formula: 62% absorption, 2.0% yeast, 2.0% salt, and 4.0% sugar.

TABLE I—(*Continued*)

Sample	Treatment	Dough quality	Volume	Crumb color	Grain and texture
SODIUM p-CHLOROMERCURICBENZOATE					
Stfd. stght.	None	Elastic	100	97	100
Stfd. stght.	0.0005%	Elastic	100	97	100
Stfd. stght.	0.0010%	Elastic	101	97	100
Stfd. stght.	0.0020%	Elastic	101	97	100
Stfd. stght.	0.0030%	Elastic	97	97	100
POTASSIUM BROMATE					
Stfd. stght.	None	Elastic	100	97	100
Stfd. stght.	0.0005%	Elastic	107	97	101
Stfd. stght.	0.0010%	Elastic	107	98	101
Stfd. stght.	0.0020%	Slightly tough	113	98	100
Stfd. stght.	0.0030%	Tough	119	98	99
POTASSIUM IODATE					
Stfd. stght.	None	Elastic	100	97	100
Stfd. stght.	0.0005%	Elastic	104	97	101
Stfd. stght.	0.0010%	Slightly tough	105	98	102
Stfd. stght.	0.0020%	Very tough	104	98	101
Stfd. stght.	0.0030%	Very tough	106	98	100

be determined what the particular compound is and whether or not it is enzymatic in nature. There is a fairly strong reducing potential in a fermenting dough and in bread itself. Olcott and Fraenkel-Conrat (17) have found that cysteine reacts with pyruvic acid in an acid solution. This may explain, if sulfhydryl compounds are present, why a positive test for sulfhydryl cannot be demonstrated in a fermenting dough. The fact that, roughly, half of the optimum dosage of bromate still remains at the end of the proof period and is reduced completely to bromide only during the first 15 minutes the dough is in the oven is definite evidence that reducing substances are liberated when bread is baked. Further proof that sulfhydryl groups are liberated is provided by titrations of a centrifuged water extract of bread, adding excess o-iodosobenzoic acid and back titration with thiosulfate. One gram of untreated bread used 0.101 ml. of 0.001 M o-iodosobenzoic acid, whereas one gram of bread made from the same flour treated with either 5 mg. % of bromate or 5 g. nitrogen trichloride/cwt. used 0.063 ml. of 0.001 M o-iodosobenzoic acid, or 38% less of the reagent.

The Disulfide Group

The disulfide group is our next consideration. Flour contains from 0.2 to 0.4% cystine and, so far as is known, cystine is the only compound containing the S–S group present in flour. Cystine, R–S–S–R, can be oxidized to RSOH, RSO_2H, and RSO_3H. Likewise, it can be reduced. It is probable that some, if not all, of the disulfide groups of the proteins of flour form cross linkages between polypeptide chains, as this structural formula illustrates (18). These cross linkages undoubtedly exert a profound influence on the physical properties of gluten. Insulin and the proteins of hair and wool are thought to possess similar disulfide bridges. The function of these cross linkages is to strengthen the protein and to suppress its plastic flow.

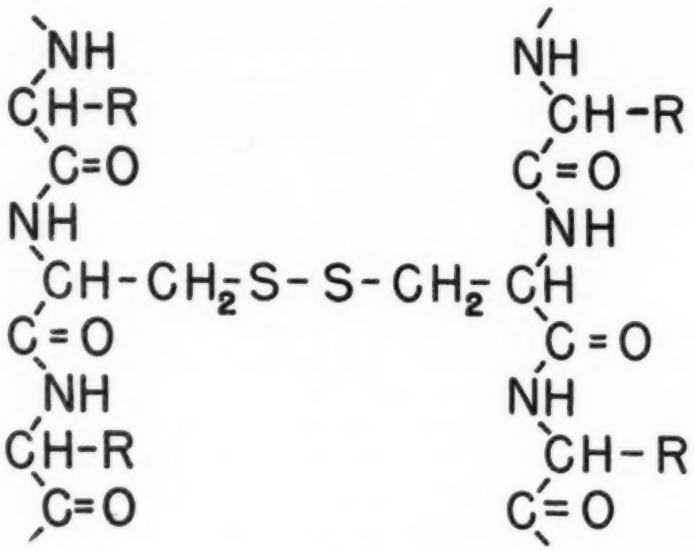

FIG. 1.

Effect of Reducing Agents on the Disulfide Group. From work done on other proteins, we can visualize what might happen when reducing agents, such as sulfides, dithionites, and thiol compounds, are added to flour or to gluten. We know that the physical properties of the dough are profoundly affected. The dough becomes soft, sticky, and, with increasing amounts of reducing agents and sufficient time, almost fluid. This behavior may well be caused by a rupture of some of the disulfide cross linkages, whereby there is a less rigid protein structure and more

slippage between the peptide chains. In the case of sulfhydryl compounds or thioglycollic acid, each disulfide linkage forms two sulfhydryl groups. Other reagents, such as sulfites, bisulfites, and cyanides, react with the disulfide group to form one sulfhydryl group.

Effect of Reducing Agents on Cystine. We have measured the loss in cystine on the addition of 5 mg. % of sodium sulfite to the gluten washed from a straight grade of flour prior to hydrolysis and found a loss of 19%, as compared with the untreated flour.

Similarly, sodium cyanide allowed to react with gluten one-half hour before hydrolysis caused a 26% loss of cystine. Cyanides are known to affect flour in a manner similar to reducing agents such as sulfites and sulfhydryl compounds. Many of you have had experience with the poor baking characteristics and extreme greenness manifested by flour immediately after fumigation with hydrocyanic acid. If the amount of cyanide is small, this effect is rather quickly dissipated by aeration. We agree with Fraenkel-Conrat (7) that the action of cyanic acid on cystine in an acid medium is not a reduction but a hydrocyanolysis, as follows: R–S–S–R + HCN → RSH + RSCN. One mole of cysteine is produced from one mole of cystine. Here, again, the action of hydrocyanic acid is adequately explained by direct attack on the cystine of flour. Treatment of flour or the gluten washed therefrom, with other reducing agents mentioned, will cause similar losses. This is not surprising, in view of the fact that the disulfide linkage is the only group known to be reduced except under conditions that denature the protein. Olcott, Sapirstein, and Blish (16) demonstrated that gluten dispersions in dilute acetic acid can be stabilized by heating for 5 to 10 minutes at 100°C. By this treatment, the solubility characteristics of the protein are not changed appreciably, but a proteinase responsible for the instability is destroyed. Reducing agents greatly lowered the viscosity of the gluten dispersions freed from enzyme activity by heat—another indication that reducing agents act mainly directly on the protein rather than as activators of the proteolytic enzymes.

Influence of Methylene Groups on the Disulfide Linkage. A few years ago, we did some other, unpublished experiments that have a direct bearing on this problem. This work was based on papers published by Patterson *et al.* (18), Geiger *et al.* (8), and Harris *et al.* (11) on the elasticity of wool and its relation to chemical structure. Gluten (20 g.) was ground in a mortar with 7 ml. of 0.2 *M* thioglycollic acid previously adjusted to pH 4.5 with potassium hydroxide. After standing one hour at 37°C., the thioglycollic acid was poured off, the gluten

thoroughly washed and divided into two equal parts. Then one fraction was immersed in an M/10 phosphate buffer pH 8, and the other immersed in the same buffer to which 0.15 ml. of 0.002 *M* trimethylene bromide was added. Both were allowed to stand overnight and again washed. The reactions are as follows (see Fig. 2):

$$RSSR + 2HSCH_2COOH \rightarrow 2RSH + (SCH_2COOH)_2$$

$$2RSH + (CH_2)_3Br_2 \rightarrow RS(CH_2)_3SR + 2HBr$$

FIG. 2.

The alkylated gluten was considerably tougher than the control and had a more rigid structure. The cystine content of the control gluten, determined by the Sullivan method, was 4.33%, whereas the alkylated gluten with methylene groups between the sulfur atoms showed only 3.50% cystine. Thus, the introduction of methylene groups in, roughly, one-fifth of the cross linkages caused a decided change in the physical properties of the gluten.

Effect of Oxidizing Agents on Cystine. In view of the evidence that the breaking of the S–S cross linkage between peptide chains is responsible for the changes observed on treating flour with certain reducing agents, it is reasonable to suppose that oxidizing agents likewise might have an effect on the disulfide linkage. Cystine can be oxidized to sulfinic acid, disulfoxide, and sulfenic and sulfonic acid derivatives. Indeed, one of the classic methods for the measurement of cystine is based on its oxidation to cysteic acid by bromate in acid solution.

As the next step, cystine was measured on the untreated and oxidized flour, gluten, and bread. The method used for cystine was the spectrophotometric procedure described by Csonka, Lichtenstein, and Denton (6), which represents a modification of the Rossouw and Wilken-Jorden mercaptide precipitation and the Sullivan colorimetric method. In the acid hydrolysis of flour and bread, the presence of relatively large amounts of carbohydrates causes some loss of cystine. However, in all determinations on flour and bread, conditions were identical except for the presence of the improver, so the error due to the presence of carbohydrates during hydrolysis was the same. Whenever possible, cystine

determinations were made on the gluten rather than on the flour, since there is no interference due to carbohydrates.

No difference in cystine content was found between the control flour and the same flour bleached with 5 g. of nitrogen trichloride per hundredweight. Also, there was no difference in the cystine content of glutens washed from the unbleached and bleached flours. Moreover, no significant difference in cystine content was shown in the bread baked from the unbleached and bleached flours.

Since bromate does not act until a flour is doughed up, there was no point in determining cystine on bromated flour or gluten washed therefrom. Because of the "oven effect" of bromate, we measured cystine on the bread baked from bromated and unbromated flour. Table II shows that neither nitrogen trichloride nor bromate had any effect on the cystine content of the flour, the gluten, or the bread baked from the flour.

TABLE II

CYSTINE CONTENT OF UNTREATED AND TREATED FLOUR AND BREAD

Sample	Cystine (dry basis) %
100% Straight flour, untreated (hydrolysis of flour)	0.21
100% Straight flour, untreated (hydrolysis of gluten)	0.29
100% Straight flour, 5 g. NCl_3 per cwt.	0.32
Bread from Straight flour untreated	0.24
Bread from Straight flour 5 g. NCl_3 per cwt.	0.24
Bread from Straight flour untreated	0.21
Bread from Straight flour 0.005% $KBrO_3$	0.20

We conclude from these experiments that, although cystine is affected by reducing agents, the action of improvers cannot be explained by their attack on the disulfide group of cystine.

Improving Action of l-Cystine. Free cystine could not be expected to have the same action as the cystine present in peptide linkage. Nevertheless, since cystine will oxidize sulfhydryl groups and is, itself, reduced in the reducing medium existing in a fermenting dough, it was of some interest to find out how varying amounts of l-cystine would affect flour in comparison with its reduced form, cysteine, and its oxidation product, cysteic acid. Table III illustrates the results.

The well-known deleterious effect of cysteine illustrates the behavior of sulfhydryl compounds. At lower levels, the action of cysteine is never as marked as that of the cysteine-containing tripeptide, glutathione. Much to our surprise, l-cystine was found to be an excellent improver. With the particular flour employed, 0.04% cystine proved to be the optimum amount and showed a loaf volume increase of 30%. With in-

creasing amounts of this amino acid, the dough becomes tougher—another characteristic indication of oxidation. Cysteic acid, one of the oxidation products of cystine, showed a slight improving effect, but this was negligible when compared to l-cystine itself. Cystine either oxidizes a sulfhydryl compound present in dough or is preferentially reduced during fermentation or in the oven, as compared to the sulfur present in peptide linkages.

TABLE III

IMPROVING ACTION OF L-CYSTINE ON STRAIGHT GRADE FLOUR [1]

Sample treatment	Dough quality	Loaf volume	Grain and texture	Crumb color
		%	%	%
None	Elastic	100	100	97
0.02% Cysteine—HCl	Soft, sticky	88	97	97
0.01% l-Cystine	Elastic	112	101	98
0.02% l-Cystine	Elastic	112	101	98
0.03% l-Cystine	Elastic, slightly tough	117	100, Sl. open	98
0.04% l-Cystine	Elastic, slightly tough	130	99	99
0.06% l-Cystine	Elastic, tough	128	99	99
0.02% Cysteic acid	Elastic	100	100	97
0.04% Cysteic acid	Elastic	104	100	98
0.05% Cysteic acid	Elastic	102	100	98
0.06% Cysteic acid	Elastic	103	100	98

[1] The sample used was a spring wheat straight grade flour containing 13.80% protein and 0.48% ash. One pound loaves were baked by the straight dough method using the following formula: absorption 59½%, yeast 2%, salt 2%, sugar 4%.

The Indole Group

In some preliminary work done last year on the action of nitrogen trichloride on various pure amino acids, one that seemed to be seriously affected was l-tryptophane. By treatment of this amino acid with high dosages of nitrogen trichloride, extreme color changes were produced and we found it possible to knock off the indole nucleus. These experiments suggested the possibility that this group might be involved in the mechanism of the oxidation and reduction in flour.

Effect of Plant Hormones. Certain plant hormones, such as beta-indole acetic acid, have been found in the endosperm of wheat (9, 10). We tried both beta-indole acetic acid and beta-indole proprionic acid, in amounts ranging from 3 to 60 mg. %, in baking tests with and without bromate. Changes in dough-handling properties and in grain and texture were noted, but there were no startling differences, as compared to the control.

Tryptophane Determinations. As a further check, we measured the tryptophane content of the untreated straight grade flour, the same flour bleached with nitrogen trichloride, and the bread made from the unbleached and bleached flours. Also, the loaves made from the untreated and bromated flours were analyzed for tryptophane. The Horn and Jones method (12), in which a papain digestion is employed for the hydrolysis, was used for all determinations. Results are summarized in Table IV.

TABLE IV

TRYPTOPHANE CONTENT OF UNTREATED AND TREATED FLOUR AND BREAD

Sample	Tryptophane (dry basis) %
100% Straight flour untreated	0.15
100% Straight flour 5 g. NCl_3 per cwt.	0.15
Bread from untreated straight	0.18
Bread from NCl_3 treated straight	0.18
Bread from untreated straight	0.18
Bread from bromated straight (0.005% $KBrO_3$)	0.18

As can be seen, there is no difference in the tryptophane content of flour or bread on treatment with common maturing agents. Apparently, the indole group is not significantly involved in the changes in flour which occur on oxidation. Moreover, l-tryptophane, itself, in amounts of from 0.02 to 0.06%, had no effect on the baking characteristics of flour.

The Thio-ether Group

Another group that could be affected by the usual maturing agents is the SCH_3 group contained in methionine. It is clearly recognized that free amino acids would not necessarily react with improvers in the same manner as if they were in peptide linkage. Nevertheless, it was thought that such an approach might provide a clue to the problem. The sulfur of thio-ethers, such as methionine, has a residual affinity which enables it to add halogens, alkyl halides, and metal salts. These addition compounds are called sulphonium salts. Like other thio-ethers, methionine can react with iodine to form a periodide. By changing the pH, iodine can be liberated. It is possible that compounds such as nitrogen trichloride could act on methionine, forming a ring structure by splitting out a molecule of water or a labile addition compound. In a recent publication, Bentley *et al.* (4) stated that methionine, mixed with starch at 14% moisture, is unique in its capacity to react with nitrogen trichloride of which it takes up one-third of its weight. These investigators

believe that gluten and other reactive proteins contain a number of centers, probably of several types. But of these centers, only one, which may occur at several places in the molecule, is essential for the production of the toxic derivative formed on treatment of certain proteins with nitrogen trichloride. Methionine is thought to be associated with this center.

Determination of Methionine. The methionine content of untreated, nitrogen trichloride treated and bromated flours and the bread made from them was measured by the colorimetric method recently described by Horn, Jones, and Blum (13), using the papain digestion procedure. Acid hydrolysis of flour, gluten, and bread gave low results in our hands, although Horn, Jones, and Blum obtained good checks between the acid hydrolysis and enzyme digestion methods on a wide variety of proteins and protein-containing substances.

TABLE V

METHIONINE CONTENT OF UNTREATED AND TREATED FLOUR AND BREAD

Sample	Methionine (dry basis) %
100% Straight, untreated	0.38
100% Straight, 5 g. NCl_3 per cwt.	0.38
Stuffed Straight, untreated	0.34
Stuffed Straight, 25 g. NCl_3 per cwt.	0.32
Bread from 100% Straight, untreated	0.36
Bread from 100% Straight, 5 g. NCl_3 per cwt.	0.32
Bread from 100% Straight, untreated	0.36
Bread from 100% Straight, 0.005% $KBrO_3$	0.37

Results on unbleached and nitrogen trichloride treated flours and on breads made from untreated, bleached, and bromated flours are shown in Table V. There is no significant difference in the methionine content of either the flours or the breads and we are forced to conclude that, if the thio-ether group of methionine is involved on oxidation by these improvers, it must be by a change in the stereochemical configuration of the molecule or by the formation of some labile addition or ring compound that is easily hydrolyzed to its original form. The toxic derivative formed with nitrogen trichloride is a special case and probably only occurs when the protein is at or near its isoelectric point. Further work will be done on the problem, to verify this conclusion. When dl-methionine was added to an untreated flour, in amounts of 0.02, 0.04, and 0.06%, no significant improvement was noted. The breads containing methionine, however, all had a most disagreeable odor.

Mode of Action of Different Improvers

In 1940 (22) we reported that no two improvers manifest their results in quite the same manner. Thus, nitrogen trichloride, sodium chlorite, chlorine, and chlorine dioxide act immediately on flour and their effects are apparent in comparing the physical properties of the glutens washed from the unbleached and the bleached flours. Persulfate, bromate, and iodate, if added to the water in which a flour is doughed up, cause only a slight change in the feeling of the gluten and this is manifested in a slightly softer, rather than a tougher, feel only after several hours standing. In a dough containing yeast, iodate shows its effect more quickly than bromate and is evident in the dough taken from the mixer. Bromate needs a more acid medium for its maximum oxidizing effect than either iodate or persulfate. Persulfate, nitrogen trichloride, chlorine, and chlorine dioxide are more effective in a relatively short fermentation period, whereas bromate and iodate show their maximum effect on a long fermentation time as well. Persulfate, iodate, bromate, and iodoacetic acid are slightly reduced by the glutathione and other reducing substances present in yeast at the mixing stage. When the optimum level of bromate is used for any flour, a large proportion remains at the end of the proofing period and is reduced to bromide only in the oven.

At the recent bromate hearing, we emphasized a fact that has been overlooked generally by cereal chemists. In the literature and in laboratory control work, optimum bromate response of flours usually is reported based on baking experiments conducted by adding a standard solution to the water-yeast mixture. The optimum amount of bromate determined in this manner is far too high and, if this quantity is added to the flour in question, overoxidation results. When bromate is added at the mill, the amount should always be determined by tests in which varying amounts of this improver are added directly to the flour. If the amount of flour available is so small that accuracy cannot be achieved in this manner, a conversion factor should be employed when the improver is added to the liquid.

In spite of the fact that improvers have different modes of action, it is probable that they all act on the same compound and that this compound contains the sulfhydryl group.

Summary

The present status of our knowledge on the mechanism of oxidation and reduction of flour is woefully incomplete.

Experimental evidence would seem to justify the conclusion that reducing agents attack the disulfide group of cystine. Some of these groups may well form cross linkages between the peptide chains. At least, this concept makes clearer the physical effects observed on reduction.

Oxidizing agents that function as improvers have no effect on cystine or tryptophane. If the thio-ether group of methionine is affected by nitrogen trichloride or bromate, an unstable compound that can revert to its original form must be produced since we could find no difference in the methionine content of untreated and oxidized flours and breads.

It is highly probable that the compound oxidized contains the sulfhydryl group and the evidence for this statement is twofold. First, glutathione has been found in the germ and feed fractions of wheat and, therefore, it or some cysteine-containing peptide may be present in flour and, second, many reagents known to act on the sulfhydryl group are improvers. Some of these reagents are not oxidizing agents but alkylating agents known to be quite specific for the detection of sulfhydryl groups. Yet no sulfhydryl compound has been isolated from the protein fraction that is primarily responsible for the beneficial effect of improvers. Little mention has been made of the so-called proteolytic theory. It may be that the sulfhydryl compound present in flour is an enzyme, but, until it is isolated and proved to be effective in the amounts in which it is present in flour, the problem remains unsolved. The fact that papain behaves, in some respects, similarly to the unknown compound, in that it is activated by reducing agents and inactivated by improvers, is no valid proof of the proteolytic theory. Reasoning by analogy is useful, but does not constitute sufficient scientific evidence.

Our efforts toward the solution of this problem will continue, in an attempt to isolate the sulfhydryl compound present in flour. Until this is accomplished, the mechanism of oxidation and the beneficial effects observed on aging and treating flour with maturing agents cannot be satisfactorily explained.

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AUTHORS

SUBJECTS